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CONTENTS OF VOLUME 48

JULY, 1931. NUMBER 1

PAGE

Experimental Nephrotic Edema. Louis Leiter, M.D., with the Technical Assistance of M. Eleanor Blish, S.B., and Dorothy R. Gaston, Ph.D., Chicago 1

Is Poliomyelitis a Disease of the Lymphatic System? Montrose T. Burrows, M.D., Pasadena, Calif..... 33

Experimental Edema. Samuel A. Shelburne, M.D., Dallas, Texas, and William C. Egloff, M.D., Boston..... 51

Insulin: Some Effects on Normal Rabbits Protected from Hypoglycemia by Ingestion of Dextrose. Robert F. Loeb, M.D.; Emily Guild Nichols, and Beryl H. Paige, New York..... 70

Acute Toxic Hepatitis (Acute Yellow Atrophy) Due to Cinchophen: Report of a Case. K. K. Sherwood, M.D., Redmond, Wash., and H. H. Sherwood, M.D., Kirkland, Wash..... 82

Essential Hypertension: The Diastolic Blood Pressure; Its Variability. David Ayman, M.D., Boston..... 89

Cinchophen Oxidation Test of the Function of the Hepatic Cells. S. S. Lichtman, M.D., New York..... 98

Physostigmine Salicylate in the Treatment of Exophthalmic Goiter: Observations on Two Hundred Cases. Israel Bram, M.D., Philadelphia..... 126

Staining of Reticulocytes by Brilliant Cresyl Blue: Influence of Solutions of Substances. Clark W. Heath, M.D., and Geneva A. Daland, S.B., Boston 133

Paravertebral Injections of Alcohol for the Relief of Cardiac Pain: A Review of Experience to Date and a Report of Nine Cases. Robert L. Levy, M.D., and Richmond L. Moore, M.D., New York..... 146

Book Reviews..... 169

AUGUST, 1931. NUMBER 2

The Relation of Achlorhydria to Pernicious Anemia. Eli Moschcowitz, M.D., New York..... 171

Circulation in Arteriovenous Aneurysm: Before and After Operation. Carter Smith, M.D., Atlanta, Ga..... 187

Malignant Nephrosclerosis: Pathogenesis. Phillip F. Shapiro, M.D., Chicago 199

Body Types in Women of Infertile Constitution: The Value of Surface Area Proportions as an Anthropometric Method of Differentiation. Samuel Gordon Berkow, M.D., Perth Amboy, N. J..... 234

Inflammation: A Protective Mechanism. Valy Menkin, M.D., Boston..... 249

Mechanism of Production of Subauricular Beats by Digitalis Bodies. Harry Gold, M.D.; Abraham Lieberson, A.B., and Ben Gelfand, M.D., New York..... 262

The Relation Between the So-Called Renal Lesions of Plasmapheresis in Dogs and Contracted Kidneys in Man. Louis Leiter, M.D., Chicago..... 286

Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus: IV. Tolerance Tests for Dextrose. A. Maurice Wakeman, M.D., and Clare A. Morrell, M.D., New York..... 301

The Rôle of Artichokes in the Diet of the Diabetic Patient. Hermann B. Stein, M.D.; Bernard B. Longwell, A.B., and Robert C. Lewis, Ph.D., Denver 313

Effects of Treatment with Oxygen in Cardiac Failure. Alvan L. Barach, M.D., and Dickinson W. Richards, Jr., M.D., New York..... 325

Book Reviews..... 348

CONTENTS OF VOLUME 48

SEPTEMBER, 1931. NUMBER 3

PAGE

Exophthalmic Goiter: The Development of Refractoriness to Iodine. Willard Owen Thompson, M.D., and Phebe K. Thompson, M.D., Chicago..... 351

Congestive Heart Failure: X. The Measurement of Ventilation as a Test of Cardiac Function. T. R. Harrison, M.D.; F. C. Turley, M.D.; Edgar Jones, M.D., and J. Alfred Calhoun, M.D., Nashville, Tenn..... 377

The Staff Count: Its Importance in Acute Infectious Disease. Arthur Weiss, M.D., New York..... 399

Unexplained Gastric Anacidity. W. Scott Polland, M.D., and Arthur L. Bloomfield, M.D., San Francisco..... 412

Metabolism in Pneumonia. Isidor Greenwald, Ph.D., New York:
II. The Mechanism of the Retention of Chloride in Pneumonia..... 418
III. The Excretion of Anions and of Total Base in Pneumonia..... 440

The Significance of Cerebrospinal Fluid Sugar. Ernest F. Wahl, M.D., Thomasville, Ga..... 446

Native Complement in Hyperthyroidism. N. Novick, Washington, D. C.... 462

The Respiratory Quotient: Its Use in the Diagnosis of Diabetes Mellitus. Max Wishnofsky, M.D., and Charles S. Byron, M.D., Brooklyn..... 470

The Significance of the Potassium-Calcium Ratio and of the Inorganic Phosphorus and Cholesterol of the Blood Serum in Arterial Hypertension. Alfred A. Weinstein, A.B., and Soma Weiss, M.D., Boston..... 478

Thrombo-Angiitis Obliterans (Buerger): VI. Chemistry of the Blood. Mae Friedlander, Ph.D., and Samuel Silbert, M.D., New York..... 500

Cutaneous Lesions Associated with a Deficiency in Vitamin A in Man. Chester N. Frazier, M.D., and Ch'uan-K'uei Hu, M.D., Peiping, China.. 507

Calcium and Phosphorus Metabolism in a Case of Nontropical Sprue with Associated Tetany. Alexander Marble, M.D., and Walter Bauer, M.D., Boston 515

Book Reviews..... 533

OCTOBER, 1931. NUMBER 4

The Treatment for Secondary Anemia: A Study of the Results in One Hundred and Twenty-Six Cases. Chester S. Keefer, M.D., Boston, and Chi-Shih Yang, M.D., Peiping, China..... 537

Metabolism in Myotonia Atrophica: Report of a Case. Sergius Morgulis, Ph.D., and Alexander Young, M.D., with the Assistance of J. K. Miller and E. Janecek, Omaha..... 569

Tuberculous Meningitis with Syphilitic Meningitis Terminating in Recovery: A Review of the Literature. David W. Kramer, M.D., and B. B. Stein, M.D., Philadelphia..... 576

The Cause of Exophthalmic Goiter: A Biochemical Hypothesis. William S. Reveno, M.D., Detroit..... 592

The Elimination of Phenolsulphonphthalein by the Kidney: The Influence of Pathologic Changes in the Liver. J. P. Hanner, M.D., and G. H. Whipple, M.D., Rochester, N. Y..... 598

Tumors of the Suprarenal Gland with Special Reference to Carcinoma of the Cortex: Report of a Case. Jacob Meyer, M.D., and Gerald Frumess, M.D., Chicago..... 611

Tumors of the Heart and Pericardium: Pathology, Symptomatology and Report of Nine Cases. Wallace M. Yater, M.D., Washington, D. C.... 627

Functional Aspects of Regenerated Hepatic Tissue. T. L. Althausen, M.D., San Francisco..... 667

Normal Asymmetry and Unilateral Hypertrophy. George Halperin, M.D., Chicago 676

PAGE

Isolated Congenital Dextrocardia: Report of Two Cases with Unusual Electrocardiographic Findings; Anatomic, Clinical, Roentgenologic and Electrocardiographic Studies of the Cases Reported in the Literature (to Be Concluded). S. S. Lichtman, M.D., New York 683

Book Reviews 718

NOVEMBER, 1931. PART I. NUMBER 5

The Causation and Prevention of Congenital Heart Disease. William D. Reid, M.D., Boston 721

Tuberculosis in Medical and College Students. H. W. Hetherington, M.D.; F. Maurice McPhedran, M.D.; H. R. M. Landis, M.D., and Eugene L. Opie, M.D., Philadelphia 734

Infective Neuronitis: Report of a Case with Autopsy Observations. Harry G. Jacobi, M.D., New York 764

The Cutaneous Histamine Reaction as a Test for Collateral Circulation in the Extremities. G. de Takáts, M.D., Chicago 769

Intestinal Oxygenation in Idiopathic Ulcerative Colitis: Preliminary Report. Joseph Felsen, M.D., New York 786

Agranulocythemia: Report of a Case with Complete Antemortem and Postmortem Records. C. Victor Richards, M.D., Baltimore 793

The Dextrose Level of the Arterial and Venous Blood in Arthritis. Robert M. Stecher, M.D., and Harold J. Jeghers, B.S., Cleveland 801

Determination of Plasma Volume:

I. The Dye Method. Samuel Graff, B.S., and H. T. Clarke, D.Sc., New York 808

II. The Rate of Dye Mixing. S. Graff, B.S.; D. A. D'Esopo, M.D., and A. J. B. Tillman, M.D., New York 821

Blood Amylase in Relation to Disease of the Pancreas: Further Observations. Robert Elman, M.D., St. Louis 828

I. Goldstein's Heredofamilial Angiomatosis with Recurring Familial Hemorrhages (Rendu-Osler-Weber's Disease). Hyman I. Goldstein, M.D., Camden, N. J 836

Isolated Congenital Dextrocardia: Report of Two Cases with Unusual Electrocardiographic Findings; Anatomic, Clinical, Roentgenologic and Electrocardiographic Studies of the Cases Reported in the Literature (Concluded). S. S. Lichtman, M.D., New York 866

Book Reviews 904

NOVEMBER, 1931. PART II. NUMBER 5

The Influence of Solar Rays on Metabolism, with Special Reference to Sulphur and to Pellagra in Southern United States. James H. Smith, M.D., Richmond, Va 907

DECEMBER, 1931. NUMBER 6

Sympathetic Activity in Certain Diseases, Especially Those of the Peripheral Circulation. W. J. Merle Scott, M.D., and John J. Morton, M.D., Rochester, N. Y 1065

Electrocardiographic Studies of the Effect of Anaphylaxis on the Cardiac Mechanism. Leo H. Criep, M.D., Pittsburgh 1098

Subacute Meningococcal Endocarditis. Norman B. Gwyn, M.D., Toronto, Canada 1110

Effects of Exercise on Experimental Cardiac Infarction. Don C. Sutton, M.D., and Milton D. Davis, M.D., Chicago 1118

Primary Carcinoma of the Lungs: Pathognomonic Signs in the Diagnosis. Jacob Polevski, M.D., Newark, N. J 1126

PAGE

The Use of Amidopyrine in Rheumatic Fever. Mark P. Schultz, M.D., New York....1138

Pigment Metabolism and Destruction of Blood in Addison's (Pernicious) Anemia. R. F. Farquharson, M.B.; H. Borsook, M.B., and A. M. Goulding, M.D., Toronto, Canada....1156

Brucella Infection of Brief Duration: Report of a Case. Hugh R. Leavell, M.D.; Mary Poston, and Harold L. Amoss, M.D., Durham, N. C....1186

The Endermic Reaction in Brucella Infections. Hugh R. Leavell, M.D., and Harold L. Amoss, M.D., Durham, N. C....1192

Peptic Ulcer: Association with Pulmonary Tuberculosis. Mills Sturtevant, M.D., and Louis Lawrence Shapiro, M.D., New York....1198

Congestive Heart Failure: XI. The Effect of Digitalis on the Dyspnea and on the Ventilation of Ambulatory Patients with Regular Cardiac Rhythm. T. R. Harrison, M.D.; J. Alfred Calhoun, M.D., and F. C. Turley, M.D., Nashville, Tenn....1203

Mechanisms of the Contraction and Evacuation of the Gallbladder. Lathan A. Crandall, Jr., M.D., Ph.D., Chicago....1217

Prophylaxis of Postoperative Pneumonia by Oral Hygiene: A Preliminary Report. Sigmund W. A. Franken, D.D.S., New York....1225

Azotemia with Normal Kidneys Found Post Mortem: Possible Cause; Low Blood Pressure After Operation. Theodore S. Evans, M.D., New Haven, Conn. 1231

Effect of Ingestion of Water and of Dextrose Solution on Emptying Time of Normal Stomach. Haig H. Kasabach, M.D., New York....1237

Book Reviews....1241

General Index....1245

JUL 27 1931

VOLUME 48 NUMBER 1

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CONTENTS OF PREVIOUS NUMBER

JUNE, 1931. NUMBER 6

Hypoglycemia: The Clinical Syndrome, Etiology and Treatment; Report of a Case Due to Hyperinsulinism. George D. Gammon, M.D., Philadelphia, and William C. Tenery, M.D., Waxahachie, Texas.

The Effect of High Altitudes on the Cholesterol, Lecithin and Fatty Acids in the Plasma of Healthy Men. Gulli Lindh Muller, M.D., and John H. Talbott, M.D., Boston.

A Biometrical Analysis of Two Thousand, Five Hundred and Sixty-Two Complete Examinations of the Blood. Edgar T. Herrmann, M.D., St. Paul.

Effect of Intrathoracic Pressure on Arterial Tension. Eli Grimes, M.D., Des Moines, Iowa.

The Value of Determinations of the Iron Content of Whole Blood. William P. Murphy, M.D.; Ralph Lynch, M.D., and Isabel M. Howard, Boston.

Pernicious Anemia with Fatal Termination During a Liver Diet: Report of Three Cases. James B. Carey, M.D., Minneapolis.

Tuberculous Infection: Attempts to Prevent It by Subcutaneous Vaccination with B C G. Johannes Heimbeck, Oslo, Norway.

Primary Carcinoma of the Duodenum: Report of Four Cases, with a Review of the Literature. Jacob Meyer, M.D., and David H. Rosenberg, M.D., Chicago.

Acute Isolated Myocarditis, with Report of a Case. Clarence E. de La Chapelle, M.D., and Irving Graef, M.D., New York.

IV. Coronary Sinus Rhythm: Rhythm Subsequent to Destruction by Radon of the Sino-Auricular Nodes in Dogs. Milton C. Borman, M.D., Milwaukee, and Walter J. Meek, Ph.D., Madison, Wis.

The Appearance of Histiocytes in the Peripheral Blood. William Dameshek, M.D., Boston.

Book Reviews.

General Index.

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Archives of Internal Medicine

VOLUME 48 JULY, 1931 NUMBER 1

EXPERIMENTAL NEPHROTIC EDEMA *

LOUIS LEITER, M.D.

WITH THE TECHNICAL ASSISTANCE OF

M. ELEANOR BLISH, S.B., AND DOROTHY R. GASTON, PH.D.

CHICAGO

Richard Bright [1] was well aware of the effects of prolonged albuminuria on the serum proteins, because he actually obtained quantitative analytic data with the help of friends who were chemists. He believed that the thin, watery plasma filtered out into the tissue spaces readily, thus producing dropsy. It is obvious that he considered renal edema as uniform in type and in causation. For almost a hundred years clinicians and physiologists failed to distinguish between the different forms of dropsy occurring in Bright's disease and sought to explain them all on a single basis. The experimental work on edema reflected accurately the prevailing clinical theories. Thus Bright's theory of hydremia as the cause of edema was put to the experimental test by Cohnheim and Lichtheim [2] and found wanting. Similarly, the rôle of oliguria and hydremic plethora defended by Bartels [3] and Grainger Stewart [4] was more or less definitely disposed of by the classic experiments of Cohnheim and Lichtheim,[2] Magnus [5] and others. There remained only one unassailed, and apparently unassailable, universal theory of renal edema—Senator's [6] conception of increased permeability of the capillaries secondary to toxic injury, expressing itself in the glomerular capillaries by albuminuria and anatomic renal disease, and in the cutaneous and serous capillaries by edema. The postscarlatinal nephritis with edema was the keystone of this theory. There was ample experimental support for the association of edema with capillary injury. The work of Cohnheim and Lichtheim,[2] Magnus,[5] Richter,[7] Georg-

* Submitted for publication, Sept. 14, 1930.

* From the Lasker Foundation for Medical Research and the Department of Medicine of the University of Chicago.

1. Bright, Richard: Reports of Medical Cases, London, 1827, vol. 1.

2. Cohnheim, J., and Lichtheim, L.: Virchows Arch. f. path. Anat. **69**:106, 1877.

3. Bartels, C.: Handbuch der speziellen Pathologie und Therapie, Leipzig, F. C. W. Vogel, 1875, vol. 9.

4. Stewart, Grainger F.: Practical Treatise of Bright's Disease of the Kidneys, Edinburgh, 1871.

5. Magnus, R.: Arch. exper. Path. u. Pharmakol. **42**:250, 1899.

6. Senator, H.: Berl. klin. Wchnschr. **32**:165, 1895.

7. Richter, P. F.: Berl. klin. Wchnschr. **42**:384, 1905; Deutsche med. Wchnschr. **36**:1737, 1910.

opulos,[8] Heineke and Meyerstein[9] and Pearce[10] clearly demonstrated the dependence of edema on damage to the capillaries by such irritants as heat, iodine, arsenic, chloroform, metabolic products in a dying animal, snake venoms and especially uranium nitrate. The analogy between the experimental edemas and the dropsy of Bright's disease seemed very close.

It is a curious fact that it did not seem to occur to any one of the numerous investigators of this problem to compare human and experimental edema fluids in a quantitative manner, although scattered analyses were published on one or the other separately. This was an important and far-reaching omission, because it delayed the proper clinical recognition of Starling's[11] contributions to the physiology of fluid exchange for about twenty years. Starling[12] had shown in 1896 that the colloid osmotic pressure of the plasma proteins was a measurable force and one quantitatively adequate to effect the reabsorption of physiologic tissue fluids into the vascular capillaries. Necessary corollaries of his theory were the relative impermeability of the normal peripheral capillaries to the plasma proteins and a low concentration of protein in normal subcutaneous tissue fluids. Therefore, when Epstein[13] demonstrated by systematic quantitative analysis of edema fluids in patients with Bright's disease that in some cases the protein content was extremely low, and when he[14] later was the first to apply Starling's concept by explaining the edema of nephrotic Bright's disease as the result of marked albuminuria and decreased concentration of plasma protein, the older theory of increased permeability collapsed for nephrotic renal edema, because, with the capillaries injured and more permeable, the edema fluid should have a considerably higher protein content than was actually found. One began, therefore, to distinguish between nephrotic, low protein edema and nephritic, high protein edema.[15] The former was a true transudate or ultrafiltrate of the plasma, something approximating the cerebrospinal fluid or the aqueous humor, while the latter was much more like an inflammatory exudate.

This distinction between clinical edema fluids, when applied to the experimental edemas produced up to 1917, leads to the obvious con-

8. Georgopulos: Ztschr. f. klin. Med. **60**:411, 1906.

9. Heineke, A., and Meyerstein, W.: Deutsches Arch. f. klin. Med. **90**:101, 1907.

10. Pearce, R. M.: The Production of Edemas, Arch. Int. Med **3**:422 (June) 1909.

11. Starling, E. H.: Lancet **1**:1267, 1331 and 1407, 1896.

12. Starling, E. H.: J. Physiol. **19**:312, 1896.

13. Epstein, A. A.: J. Exper. Med. **20**:334, 1914.

14. Epstein, A. A.: Am. J. M. Sc. **154**:638, 1917.

15. Beckmann, K.: Deutsches Arch. f. klin. Med. **135**:39, 1921.

clusion that all of the earlier experimental edemas were of the exudative type, rich in protein and undoubtedly based on more or less extensive capillary damage with resulting increased permeability to the plasma proteins. Therefore, no one had really produced a nephrotic edema.

THE PROBLEM

The problem was relatively simple as it presented itself in 1925. At the suggestion of Dr. D. D. Van Slyke, work was begun on dogs with the specific aim of producing, by means of experimental hypoproteinemia, an edema fluid low in protein. If renal, cardiac and capillary damage could be excluded as significant factors in this edema, the Starling-Epstein theory of nephrotic edema would find its first direct experimental confirmation. It was recognized at the outset that an adequate supply of salt and water was an important accessory factor, for, regardless of the general tendency to edema as the result of the experimental procedure, little transudate could be formed without freely available salt and water. The most important single feature of this work was considered to be the determination of the protein content of the experimental effusions. Without such quantitative data no conclusions could safely be drawn as to the pathogenesis of the experimental edema.

Since the first preliminary publication of our results [16] the problem has expanded in various directions, and at present involves the question of experimental nephrosis,[17] that is, the whole nephrotic syndrome, the renal lesions supposed to result from plasmapheresis [18] and other phases on which no final statements can yet be made. It may be said, however, that the original simpler problem has been solved with a reasonable degree of certainty. There is no doubt now as to the relationship between low plasma proteins and a nephrotic type of edema in dogs. That the same situation may hold true for rabbits was suggested earlier by Fishberg and Fishberg,[19] but they observed no gross edema, and therefore had no material available for analysis.

METHODS

Physiologic.—Dogs that weighed between 10 and 15 Kg. were ordinarily used. They were kept on the standard diet of a mixture of bread and meat without exact quantitative control. Water was allowed ad libitum. The animals were bled

16. Leiter, L.: Proc. Soc. Exper. Biol. & Med. **26**:173, 1928.

17. Barker, M. H., and Kirk, E. J.: Experimental Edema (Nephrosis) in Dogs in Relation to Edema of Renal Origin in Patients, Arch. Int. Med. **45**:319 (March) 1930.

18. Barker, M. H.: Proc. Soc. Exper. Biol. & Med. **27**:608, 1930. Barker and Kirk (footnote 17).

19. Fishberg, E. H., and Fishberg, A. M.: Proc. Soc. Exper. Biol. & Med. **25**:296, 1928.

usually from the heart, by suction through a 3 inch gage 16 needle connected with a 250 cc. pyrex centrifuge bottle containing 20 cc. of 5 per cent sodium citrate solution as an anticoagulant. The blood was centrifugated for one hour at high speed, the plasma pipetted off as completely as possible, and the erythrocytes resuspended in the equivalent volume of a modified alkaline Locke's solution containing 7 Gm. of sodium chloride, 2 Gm. of sodium bicarbonate and 0.42 Gm. of potassium chloride per liter of distilled water. In order to save time, the erythrocytes obtained at one bleeding were not used until the next, a donor's red blood cells being injected at the first bleeding. In view of the tendency to a decrease in the volume of red cells in these experiments, donor's erythrocytes were added as necessary to bring up the volume of the cells of the injected mixture to about the normal value of from 45 to 50 per cent. Occasionally reactions were observed suggesting incompatibility of bloods, but these were so rare as to warrant the omission of routine tests for iso-agglutinins. The dogs were usually bled twice daily, and ordinarily between 400 and 500 cc. of blood would be withdrawn at each sitting. It was found advisable to begin reinjecting the warmed erythrocyte-saline suspension after the first bottle of blood had been drawn. The rest of the bleeding would be carried out while the suspension was running slowly into one of the veins of the leg. In this way serious shock was avoided, and the dogs were usually quite normal immediately after the procedure was completed. Puncture of the right side of the heart was safer than that of the left, because of the course of the coronary vessels. With a little preliminary training the animals could be bled without the use of any anesthetic or hypnotic drug. When bleeding from the external jugular veins was carried out, the dogs were kept sitting up and the head held high by an assistant, or else they were tied down on their backs while the head was allowed to project beyond the edge of the table and the snout strapped to an iron rod. An assistant compressed the vein just above the clavicle. No aseptic precautions were employed beyond ordinary cleanliness of glassware, needles and solutions. Usually 1,500 cc. of 0.85 per cent sodium chloride solution was given daily in divided doses, by stomach tube. Edema fluid was obtained from the subcutaneous tissues by puncture of the skin with a large needle, care being taken to avoid drawing blood. Gentle squeezing of the surrounding tissues would yield enough fluid for analysis. Ascitic fluid was drawn off by syringe or suction through a large needle or at autopsy. Samples of blood were usually drawn from the external jugular veins and transferred to appropriate containers.

Chemical.—The plasma proteins were estimated from the difference between the total nitrogen and the nonprotein nitrogen of the plasma, the following formula being used:

[Total nitrogen (grams per liter) — nonprotein nitrogen (grams per liter)] $\times 0.625 =$ grams of plasma protein per hundred cubic centimeters.

The Koch-McMeekin[20] digestion method was used in the determination of the total plasma nitrogen. The distillation was carried out without transfer of the digestion mixture to another container. Tenth-normal acid and alkali were used in the titrations, with alizarin sulphonate as the indicator. The nonprotein nitrogen of the plasma was also determined according to Koch and McMeekin's method up to the end of the digestion process. The ammonia nitrogen was then distilled over into fiftieth-normal sulphuric acid and titrated with fiftieth-normal sodium hydroxide, phenol red being used as the indicator.

In the fractionation of the plasma proteins into albumins and globulins, Howe's[21] method was used for the precipitation of the globulins by means of

20. Koch, F. C., and McMeekin, T. L.: J. Am. Chem. Soc. **46**:2066, 1924.

21. Howe, P. E.: J. Biol. Chem. **49**:109, 1921.

22.2 per cent sodium sulphate. The entire procedure was carried out in a thermostat held at 37 C., and an interval of three hours elapsed between the precipitation and the filtration. The water-clear filtrate was analyzed for nitrogen by the method given for total plasma nitrogen. This value was subtracted from that of the total plasma nitrogen to give the globulin nitrogen. The albumin nitrogen was obtained by subtracting the nonprotein nitrogen from the filtrate nitrogen. The factor 0.625 was used to convert nitrogen values per liter into protein values per hundred cubic centimeters.

The serum calcium was estimated by the Clark-Collip[22] modification of the Kramer-Tisdall method. The carbon dioxide content of the serum was obtained by means of the manometric procedure of Van Slyke and Neill.[23] The cholesterol of the blood was determined by Leiboff's[24] method until April 25, 1929, and from then on by the following method: Extraction of 0.2 cc. of blood by 12 cc. of a 3:1 alcohol-ether mixture was carried out in the presence of kaolin. After thirty minutes, the mixture was centrifugated and the supernatant liquid decanted into a beaker, allowed to evaporate over night and the residue extracted with chloroform. The usual colorimetric procedure based on the color developed with acetic anhydride and sulphuric acid was then completed. The standard consisted of 0.4 mg. of cholesterol in 10 cc. of chloroform.

All of the chemical determinations were done in duplicate and blank analyses on reagents were frequently made.

Anatomic.—Autopsies were performed by us on practically all of the dogs, and the various organs, with the exception of the central nervous system, were carefully examined. Histologic preparations were made from material fixed in a diluted solution of formaldehyde, U. S. P. (1:10). Unstained frozen sections of the kidneys and liver were studied with the polarizing microscope for the presence of doubly refracting lipoids (cholesterol esters). Great pains were taken to exclude confusing crytalline and amorphous precipitates, and in every instance the slide was warmed to bring out the characteristic fluid crystals. Without this precaution error is easy for the inexperienced investigator. Scarlet red was used as the stain for fat, with or without hematoxylin as a counter-stain. Paraffin or celloidin sections of various organs and tissues were prepared by standard methods and stained with hematoxylin and eosin. Blocks were cut from various portions of both kidneys, so that many sections were available for detailed scrutiny and a truly representative picture of the anatomic changes were obtainable.

RESULTS

In the preliminary report published in 1928[16] and based on successful experiments on ten dogs, it was stated that "the edema usually begins when the plasma proteins have fallen to 3 per cent or less and recedes with a rise above that level if daily bleeding is discontinued." Observations are now available on more than forty dogs with varying degrees of edema. Table 1 shows the values for the plasma proteins in relation to the beginning and the decrease in gross edema. Unfortunately, it was not possible in all instances to estimate the plasma proteins for the day on which the changes in edema occurred. It is apparent,

22. Clark, E. P., and Collip, J. B.: J. Biol. Chem. **63**:461, 1925.
23. Van Slyke, D. D., and Neill, J. M.: J. Biol. Chem. **61**:523, 1924.
24. Leiboff, S. L.: J. Lab. & Clin. Med. **10**:857, 1925.

however, that in general gross edema developed when the plasma proteins had fallen to about 3 per cent, and that definite decrease in edema usually occurred at a concentration of plasma protein between 3 and 4 per cent. Edema was never observed to begin when the plasma proteins were above 4.3 per cent and was never absent when they fell below 2.5 per cent, provided these respective levels for protein were maintained for twenty-four hours. Gross edema was chosen as the criterion in all these observations since it is something that can be seen, felt and weighed and does not require intricate calculations regarding the total water metabolism of the animal.

TABLE 1.—*The Percentages of Plasma Protein During Increasing and Decreasing Edema*

Dog	Onset or Increase of Edema	Decrease in Edema	Dog	Onset or Increase of Edema	Decrease in Edema
4	3.01	4.74*	37	2.72	4.00
10	3.40	4.62*		3.38	....
	3.82	....	38	3.58	4.25*
11	2.95	....	39	2.89	3.80
14	4.16	....	41	3.71	4.62
15	2.86	....		3.06	4.77*
16	2.71	3.57*	46	2.38	2.96*
17	2.89	2.64	47	3.70	4.14
	3.07	3.32	50	3.04	....
	2.17	2.92	53	3.36	3.88*
	1.82	3.20*	54	2.89*	4.14*
20	2.32	....	55	3.24	....
25	2.42	2.93	56	2.64	3.84*
26	2.84	....	57	2.57	3.05*
27	2.98	....		2.73	....
28	4.31†	....	69	2.78†	3.64*
32	3.94	....	70	3.08†	3.46*
33	3.09	4.03		2.14	3.76*
34	3.76	4.20	71	2.38	3.74*
	2.88	5.34*	73	3.48†	....
	3.54	3.90	86	2.48	4.33*
	3.68	4.16*		3.80†	5.66
	3.12	4.15		3.54	3.91*
35	3.42	4.17	89	2.78	5.14*
36	2.89	4.60*	90	2.04	3.44*
	2.28	3.00		2.57†	2.38*
	2.94	3.32		3.20	4.48*
	3.42	4.91*			
	3.39	3.94*			
	2.70	....			

* Twenty-four hours or more after decrease in edema occurred.
† Twenty-four hours before edema appeared.

The relation between plasmapheresis, concentration of plasma protein and edema is well illustrated in the protocols given in tables 1 to 4, and in figures 1 to 5. Whenever depletion of plasma is omitted for a single day, a prompt rise in the plasma proteins usually follows along with a definite decrease in the weight of the animal. This loss of weight is largely the expression of diuresis and disappearance of edema. The final weight reached may be considerably lower than the original control weight because of the decreased intake of food and the breakdown of tissue during the period of the experiments.

TABLE 2.—*Protocols of Dogs in Which Edema Developed as a Result of Depletion of Plasma*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.*	Total Plasma Nitrogen, Gm. per Liter	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
4	3/26/28	10.0	870	810	9.44	0.38	5.66	1,500	This experiment was partly obscured by the mistaken use of pituitary extract subcutaneously
	3/27/28	10.8	895	895	4.48	0.30	2.61	1,000	
	3/28/28	11.2	920	895	4.48	0.83	2.28	1,500	
	3/29/28	11.6	920	920	4.81	0.49	2.70	1,500	
	3/30/28	11.8	460	460	5.11	0.30	3.01	1,000	Edema of walls of abdomen and chest
	3/31/28	11.2	460	460	5.45	0.33	3.20	1,800	Edema increasing
	4/ 1/28	....	...	...	....	....	....	600	Edema receding
	4/ 2/28	10.2	870	920	7.85	0.27	4.74	1,500	No edema
10	4/30/28	10.6	1,150	1,150	8.56	0.21	5.22	1,500	Some shock
	5/ 1/28	11.0	1,275	1,275	4.96	0.41	2.84	1,500	
	5/ 2/28	11.0	810	810	5.78	0.25	3.45	1,500	Moderate shock
	5/ 3/28	11.0	1,205	1,380	....	....	....	1,500	
	5/ 4/28	....	410	460	5.85	0.40	3.40†	1,500	Edema of the legs; 25 cc. of ascitic fluid aspirated
	5/ 5/28	12.0	435	460	6.73	0.23	4.06	1,500	Edema more marked on abdominal wall
	5/ 6/28	....	...	...	....	....	....	500	Edema receding
	5/ 7/28	10.8	920	920	7.76	0.36	4.62	1,500	No subcutaneous edema; 60 cc. of ascitic fluid aspirated
	5/ 8/28	10.6	920	920	6.33	0.22	3.82	1,500	Edema returning on lower part of abdomen; 225 cc. of ascitic fluid drawn
	5/ 9/28	11.2	920	460	5.85	0.24	3.51	1,000	Increasing edema and ascites; photographed; death from acute hemopericardium
11	5/10/28	....	335	335	....	....	....	.....	
	5/11/28	11.2	360	360	10.42	0.16	6.40	1,000	
	5/12/28	11.0	385	410	9.62	0.33	5.80	1,000	Vomited about 400 cc. of saline with food
	5/14/28	9.8	820	800	8.33	0.16	5.10	1,600	
	5/15/28	10.6	920	1,000	6.73	0.22	4.07	1,500	Chills
	5/16/28	11.2	920	1,000	5.13	0.41	2.95	1,500	Edema of external genitalia, buttocks and perineum; 150 cc. of ascitic fluid aspirated
	5/17/28	11.6	895	1,000	5.12	0.21	3.07	1,500	Edema spreading over walls of abdomen and chest; photographed
	5/18/28	12.8	895	1,000	4.08	0.41	2.29	1,400	Carbon dioxide content of serum, 24.02 millimols; increasing edema of the legs and parietes; much ascites
	5/19/28	13.2	435	500	3.52	0.25	2.04	1,500	Carbon dioxide content of serum, 19.9 millimols; serum calcium, 5.92 mg.; enormous dependent edema; considerable coughing
	5/20/28	14.0	...	...	....	....	....	.....	Found dead; marked pulmonary edema; no pericardial effusion; 1,500 cc. of ascitic fluid; extreme anasarca

* In this and the following tables the figures in this column represent the theoretical volume of blood corresponding to the actual volume of packed erythrocytes, assuming a normal erythrocyte-plasma ration of 1:1.

† Blood sample taken after first dose of saline.

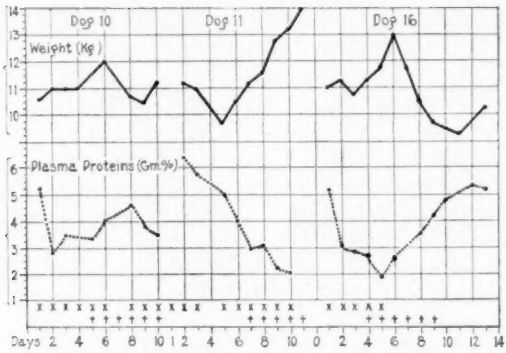

Fig. 1.—Showing the occurrence of edema and sharp gain in weight as the concentration of plasma proteins is reduced by acute plasmapheresis. The curve for dog 16 also illustrates the immediate decrease in weight due to diuresis, which occurs when the plasma proteins are allowed to increase above the critical level for edema.

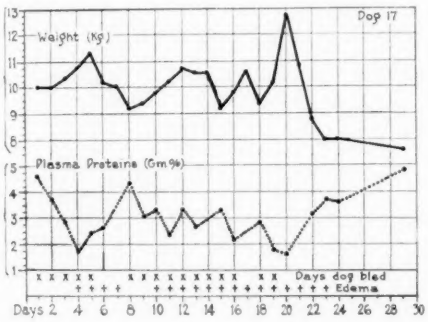

Fig. 2.—Periods of edema synchronous with levels of plasma proteins at or below 3 Gm. per hundred cubic centimeters. In each interval between periods of plasmapheresis, the weight drops rapidly as the plasma proteins are restored.

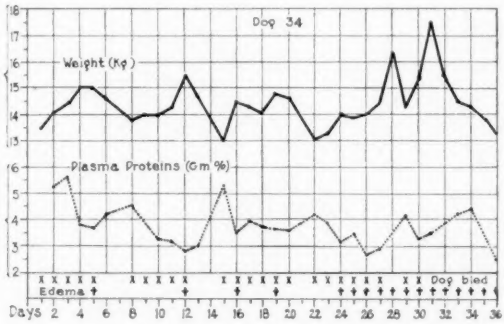

Fig. 3.—In this dog, the edema produced by plasmapheresis was exaggerated by the intravenous injection of Locke's solution administrated on the day preceding each of the last two peaks in the weight curve.

The time of the first appearance of edema is interesting because it indicates the dependence of the transudates on the reduction of the plasma proteins. In most of the dogs used earlier, edema was first observed after from three to seven days of vigorous plasmapheresis

TABLE 3.—*Protocol of a Dog with Recurrent Periods of Edema During Plasmapheresis*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.	Total Plasma Nitrogen, Gm. per Liter	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Serum Calcium, Mg. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
17	6/18/28	10.0	845	825	7.54	0.14	4.63	10.53	1,000	
	6/19/28	10.0	770	800	6.25	0.28	3.74	8.94	1,500	500 cc. of milk given
	6/20/28	10.4	770	800	4.72	0.10	2.89	7.86	1,500	Slight edema of prepubic region
	6/21/28	10.8	820	800	3.04	0.30	1.71	8.04	1,500	Carbon dioxide content of serum, 24.6 millimols; not eating; edema increasing over abdominal wall
	6/22/28	11.4	310	350	4.16	0.20	2.48	7.16	1,500	More edema, involving wall of chest
	6/23/28	10.2	...	...	4.48	0.27	2.64	8.78	1,500	Carbon dioxide content of serum, 26.6 millimols; edema receding
	6/24/28	10.1	....	....	....	....	....	....	.....	Slight edema
	6/25/28	9.2	885	850	8.00	1.02*	4.37*	9.40	1,500	No edema
	6/26/28	9.4	845	925	5.05	0.14	3.07	9.09	1,500	Chills; not eating; slight edema of lower part of abdomen
	6/27/28	9.8	845	725	5.78	0.37	3.38	9.28	1,500	Edema increasing; not eating
	6/28/28	10.2	820	700	4.01	0.16	2.41	....	1,500	More edema
	6/29/28	10.8	870	625	5.68	0.25	3.39	8.20	1,500	500 cc. of milk given; considerable edema of thighs and abdomen, spreading to wall of chest
	6/30/28	10.6	895	600	4.64	0.32	2.67	8.37	1,500	Edema unchanged
	7/ 1/28	10.6	460	275	....	....	....	....	1,000	Swelling extended to neck; general condition good
	7/ 2/28	9.2	895	825	5.53	0.22	3.32	7.94	1,500	Much less edema
	7/ 3/28	9.8	845	700	3.68	0.19	2.17	7.12	1,500	Edema increasing rapidly again
	7/ 4/28	10.6	...	...	....	....	....	....	1,500	Marked diuresis and decrease in edema
	7/ 5/28	9.4	1,330	975	4.80	0.12	2.92	8.15	1,500	Edema increasing in the afternoon
	7/ 6/28	10.2	1,230	875	3.21	0.30	1.82	7.40	.....	More edema
	7/ 7/28	12.8	...	...	2.96	0.30	1.66	6.30	1,600	Marked generalized edema and ascites; bilateral hydrothorax; photographed
	7/ 8/28	10.9	...	...	....	....	....	....	1,700	Marked diuresis; edema disappearing
	7/ 9/28	8.8	...	...	5.20	0.08	3.20	7.59	.....	
	7/10/28	8.0	...	...	6.34	0.44	3.69	8.45	.....	Trace of edema on the lower part of abdomen
	7/11/28	8.0	...	...	6.00	0.16	3.65	8.88	.....	No edema
	7/16/28	7.6	...	...	8.00	0.26	4.84	9.08	.....	

* Sample of blood drawn after first dose of saline. The value for nonprotein nitrogen is probably erroneous.

carried out without any intermission. On the other hand, in some of the later experiments relatively long periods of time elapsed before edema first appeared. This is to be explained partly on individual variations in the ability of the dog to regenerate plasma proteins and thus maintain them above the critical level, and partly on the fact that plasmapheresis was interrupted for periods of a day or two, during which considerable restoration of the plasma proteins could occur. Since there are more

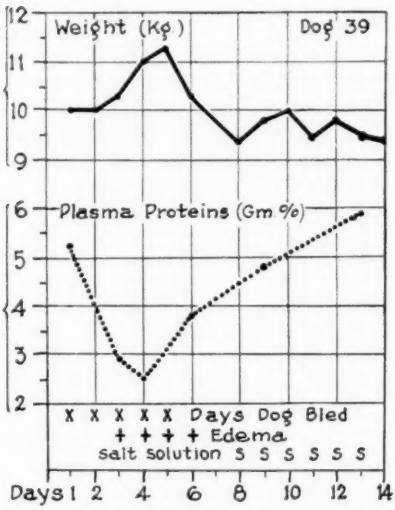

Fig. 4.—Typical edema in a dog bled from the external jugulars alone. Note the disappearance of edema after the sixth day, in spite of the continued administration of saline solution.

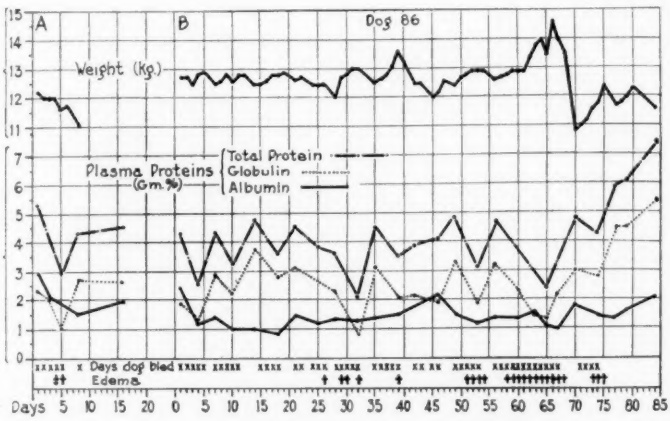

Fig. 5.—Chronic plasmapheresis is associated with periods of edema when the plasma proteins have been reduced to the edema level. In the intervals, however, edema rapidly disappears. The plasma globulin is more quickly restored than the plasma albumin.

plasma and more grams of plasma protein in a larger dog than in a smaller one, it will take considerably more bleeding to deplete the plasma and to bring on edema. If the quantity of plasmapheresis is inadequate, no edema will occur, even though daily bleeding is prac-

TABLE 4.—*Protocol of Dog with Recurrent Edema as a Result of Plasmapheresis; Also Showing the Effects of Acute Hydremic Plethora*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.	Total Plasma Nitrogen, Gm. per Liter	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
34	10/29/28	13.5	460	250	....	....	....	.....	Nc albuminuria
	10/30/28	14.1	920	895	8.63	0.08	5.35	1,000	Trace of albuminuria
	10/31/28	14.5	770	845	....	....	....	1,500	No albuminuria
	11/ 1/28	15.0	845	895	6.30	0.14	3.85	2,000	
	11/ 2/28	15.0	895	900	6.08	0.06	3.76*	2,000	Slight edema of thighs
	11/ 3/28	14.6	...	...	6.92	0.19	4.20	.....	No edema
	11/ 5/28	13.8	920	875	7.48	0.18	4.56*	1,500	Heavy trace of albuminuria
	11/ 6/28	14.0	920	875	....	...	....	1,500	No albuminuria
	11/ 7/28	14.0	920	925	5.52	0.14	3.37*	1,500	No edema; good condition
	11/ 8/28	14.3	895	900	5.44	0.23	3.26	1,500	
	11/ 9/28	15.5	870	875	4.80	0.20	2.88	1,500	Slight edema of thighs
	11/10/28	14.7	...	...	5.08	0.25	3.02	1,000	Edema unchanged
	11/12/28	13.0	1,100	1,025	8.76	0.22	5.34	1,500	No edema
	11/13/28	14.5	1,025	950	5.87	0.20	3.54	1,500	Slight edema of thighs
	11/14/28	14.3	1,000	875	6.50	0.27	3.90	1,500	No edema
	11/15/28	14.1	920	900	6.44	0.34	3.81	2,000	No edema
	11/16/28	14.8	920	800	6.15	0.27	3.68	2,000	Slight edema of thighs
	11/17/28	14.7	1,050	1,050	5.94	0.17	3.61*	2,000	No change
	11/18/28	....	.....	.....	....	....	....	.....	No edema
	11/19/28	13.1	895	900	6.85	0.20	4.16	1,500	Excellent condition; on bread and milk
	11/20/28	13.3	920	825	6.43	0.20	3.90	1,500	
	11/21/28	14.0	920	975	5.09	0.09	3.12*	1,500	Slight edema
	11/22/28	13.9	1,125	1,100	5.66	0.13	3.46*	1,500	No change; not eating
	11/23/28	14.0	1,150	1,100	4.00	0.20	2.75	1,500	Given meat; collepse after bleeding
	11/24/28	14.4	920	875	4.81	0.09	2.95*	1,500	Edema of thighs and buttocks; 2,175 cc. of Locke's solution intravenously after last bleeding
	11/25/28	16.4	...	...	....	....	....	.....	Rolls of edematous tissue along entire ventral aspect of body
	11/26/28	14.3	920	900	6.72	0.08	4.15	1,500	Still much edema
	11/27/28	15.4	820	825	5.38	0.08	3.32	1,000	Edema unchanged; 2,775 cc. of Locke's solution intravenously, increasing edema
	11/28/28	17.5	...	...	5.80	0.23	3.48	1,500	Extreme edema; dyspnea; photographed
	11/29/28	15.5	...	...	....	....	....	.....	Edema decreasing
	11/30/28	14.5	...	...	6.78	0.07	4.20	1,500	Not eating; less edema
	12/ 1/28	14.3	...	...	7.22	0.14	4.43	.....	
	12/ 2/28	13.8	...	...	....	....	....	.....	Dog quite weak; albuminuria
	12/ 3/28	13.3	...	...	4.53	0.44	2.56	.....	Slight edema; death; 2 liters of bloody fluid in abdomen, spleen full of infarets, marked pulmonary edema

* Sample of blood drawn after first dose of saline.

ticed. If at any time, however, daily depletion of plasma is carried out to the proper extent in relation to the weight of the animal, edema will inevitably develop in less than a week. This may be clearly seen in the charts of our experiments and in the published protocols of Barker and Kirk.[17]

The distribution of the edema was quite constant in the different dogs. Pitting, soft edema was first observed along the inner aspect of the thighs, and about the external genitalia and the loose tissues of the lower part of the abdomen. Some ascites usually appeared at about the same time, or even earlier. As the edema increased more of the abdominal wall became swollen, and later the anterior and lateral thoracic walls. At the same time the perineal region and thighs increased in size and edema extended down the hind legs. The fore legs were rarely involved. The face was not affected. The subcutaneous fluid shifted readily with change in position of the animal. Figures 9 to 11 illustrate the edema observed in some of the dogs.

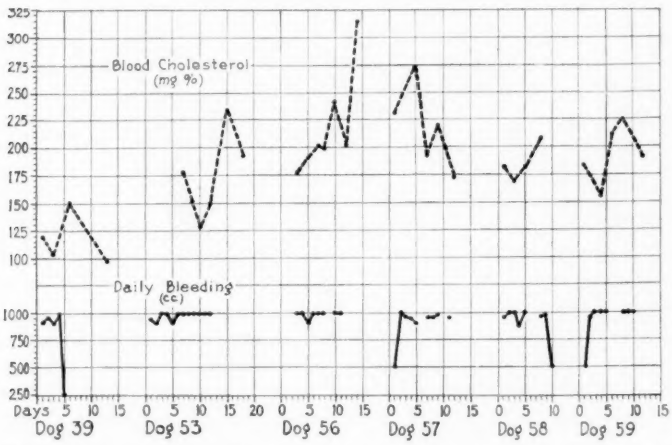

Fig. 6.—In acute plasmapheresis the blood cholesterol is quite variable, but it rises temporarily after the bleeding is discontinued.

The edema fluid obtained by puncture of the skin is perfectly clear and limpid or shows a slight opalescence. With the heat and acetic acid test, only a faint trace of protein can be demonstrated. The ascitic fluid is usually opalescent, at times definitely lactescent. On centrifugation, some of the cloudiness may clear up. This fluid ordinarily gives a more definite qualitative test for protein than the subcutaneous transudate. Chloride and bicarbonate are found in the amounts expected for transudates. The chloride content of the edema fluid is always higher, by a few per cent, than the chloride concentration in the serum.

The most significant observation is the low protein content of these experimental transudates. The figures in table 5 show that, for the most part, the concentration of protein in the edema fluids is as low as that found in normal cerebrospinal fluid. The maximum protein

content of subcutaneous fluid is 0.25 per cent, of ascitic fluid, 0.47 per cent. Previous to the preliminary report of these analyses there had been no data in the literature on the protein content of experimental nephrotic transudates. The relation between our quantitative data and those available on other experimental edemas will be discussed later.

It was recognized early in this work that the myocardial damage resulting from repeated cardiac punctures might be an important factor in the edema produced. At least, it was something to be ruled out by bleeding the animals in another way. The external jugular veins were therefore utilized in a series of dogs. Typical edema appeared in the usual time, as shown in table 6 and figure 4. The transudates were not as extensive as in the previous cardiac group of dogs, but

TABLE 5.—*The Low Protein Content in Experimental Nephrotic Edema in Dogs*

Dog	Edema Fluid	Protein, per Cent
10	Ascitic	0.04
	Ascitic	0.12*
	Ascitic	0.06
11	Ascitic	0.47
	Ascitic	0.24
	Subcutaneous	0.25
14	Ascitic	0.42
15	Ascitic	0.35
	Ascitic	0.02
	Subcutaneous	0.02
16	Ascitic	0.39*
	Ascitic	0.11*
17	Subcutaneous	0.02*
	Subcutaneous	0.05*
25	Subcutaneous	0.02
	Ascitic	0.25
32	Ascitic	0.04
	Subcutaneous	0.01
33	Ascitic	0.04
34	Ascitic	0.34
	Subcutaneous	0.04
37	Ascitic	0.02
41	Ascitic	0.09
	Ascitic	0.12
50	Ascitic	0.01*
	Ascitic	0.10*

* Only the total nitrogen was estimated on the edema fluid. The value for plasma non-protein nitrogen was used in calculating the protein content.

this difference may be partially attributed to the difficulty in bleeding the animals for more than a few days, after which thrombosis of the veins set in. These experiments were reported briefly in 1929,[25] and have found further confirmation in the independent work of Barker and Kirk,[17] who employed arterial bleeding as the method of obtaining depletion of plasma. It is an interesting fact that among the large number of dogs bled frequently from the heart not a single instance of chronic congestive heart failure has developed, in spite of severe myocardial trauma in some of the animals.

Another series of control experiments was planned to rule out the alkalinity of the modified Locke's solution used as the suspending medium for the erythrocytes. The high bicarbonate content of this solution was originally adopted in order to replace the bicarbonate lost in discarding the plasma. This solution was entirely unbuffered

25. Leiter, L.: J. Clin. Investigation **7**:493, 1929.

and therefore unphysiologic. However, in a few dogs the p_H of the serum and the total carbon dioxide content were found to be normal at the height of edema. As an added control a solution [26] was made up containing sodium chloride, sodium bicarbonate, potassium chloride, disodium acid phosphate, potassium dihydrogen phosphate and calcium chloride in physiologic proportions and carbon dioxide passed into it to bring the p_H to 7.3. This solution was kept under paraffin oil. Difficulties were encountered during its use owing to the tendency of the erythrocyte suspension to coagulate in some instances. However, several experiments were carried through to the stage of edema. This developed in the typical location and had the usual low protein content.

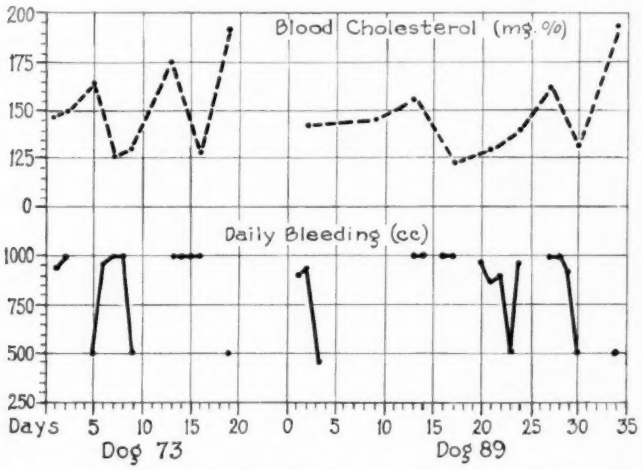

Fig. 7.—In longer experiments the blood cholesterol shows a cyclic fluctuation, the peaks occurring in the intervals between periods of plasmapheresis.

The rôle of starvation, or at least of undernutrition, presented itself next as a factor to be controlled. Although most of the dogs ate heartily throughout the course of the experiments, it could not be denied that the bleedings, twice a day, might deprive the animal of most of the absorbed elements of food. In a number of instances the blood was definitely lipemic, suggesting that the plasmapheresis had coincided with the absorptive alimentary lipemia. This occurred in spite of the fact that the dogs were usually fed late in the afternoon after the day's plasmapheresis was over. Dogs were therefore starved for a week or more, but were given 0.85 per cent saline by stomach

26. Prof. A. B. Hastings furnished us with the composition and the method of preparation and storage of this solution.

TABLE 6.—*Protocols on Dogs Bled from the External Jugular Veins Instead of From the Heart*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.	Total Plasma Nitrogen, Gm. per Liter	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
39	3/25/29	10.0	845	975	8.54	0.24	5.19	1,500	Blood cholesterol, 118 mg. per 100 cc.; blood drawn from external jugulars
	3/26/29	10.0	895	1,050	....	....	....	1,500	
	3/27/29	10.3	845	975	4.76	0.14	2.89	1,500	Edema of external genitalia and thighs
	3/28/29	11.0	920	950	4.20	0.16	2.53*	1,500	Edema spreading over walls of the abdomen and chest
	3/29/29	11.3	230	250	....	....	....	1,500	Edema increasing; jugulars closed; photographed
	3/30/29	10.3	...	...	6.30	0.22	3.80	.....	Much less edema; blood cholesterol, 152 mg.
	4/ 1/29	9.4	...	...	....	....	....	1,500	No edema
	4/ 2/29	9.8	...	...	7.91	0.21	4.81*	1,500	1,500 cc. of saline daily from 4/2 to 4/6
	4/ 6/29	9.6	...	...	9.73	0.26	5.92*	1,500	No edema since 4/1; blood cholesterol, 97 mg.
46	3/11/29	10.8	...	...	....	....	....	.....	Blood drawn from external jugulars
	3/12/29	....	460	475	....	....	....	500	
	3/13/29	10.8	920	925	7.00	0.20	4.25	1,500	
	3/14/29	10.6	920	1,050	....	....	....	1,500	
	3/15/29	10.0	895	1,000	3.78	0.24	2.22	1,500	Jugulars closing; no edema
	3/16/29	10.3	410	475	4.06	0.26	2.38	1,500	Edema of thighs and external genitalia; 40 cc. of ascitic fluid aspirated
	3/17/29	10.6	...	...	....	....	....	.....	Edema persisted unchanged
	3/18/29	10.6	...	...	....	....	....	1,500	Decrease in edema
	3/19/29	10.3	...	...	4.88	0.14	2.96	1,500	Slight edema
	3/20/29	10.3	...	...	....	....	....	1,500	No edema; saline continued daily
	3/29/29	10.4	...	...	7.63	0.19	4.68*	1,500	No edema since 3/20
47	3/18/29	12.0	845	1,000	10.50	0.16	6.46	1,500	Blood drawn from external jugulars
	3/19/29	12.4	820	925	....	....	....	1,500	500 cc. of milk given
	3/20/29	12.6	870	975	6.10	0.17	3.70*	1,500	Edema of external genitalia
	3/21/29	13.2	795	925	5.40	0.24	3.23	1,500	Edema increasing rapidly; cough
	3/22/29	13.2	230	250	....	....	....	2,500	Jugulars closed; edema unchanged
	3/23/29	12.8	...	...	6.71	0.09	4.14*	1,500	Less edema
	3/25/29	12.0	...	...	....	....	....	1,500	No edema
	3/26/29	12.0	...	...	7.56	0.16	4.63	1,500	No edema; good condition

* Sample of blood drawn after first dose of saline.

TABLE 7.—*The Plasma Proteins During Starvation*

Dog	Days of Starvation	Initial Weight, Kg.	Final Weight, Kg.	Initial Plasma Proteins, Gm. per 100 Cc.	Final Plasma Proteins, Gm. per 100 Cc.	Total Saline Given by Stomach Tube, Cc.
38	6	11.0	10.3	5.53	5.57	7,000
39	11	10.0	9.1	5.76	5.88	10,000
43	7	8.2	7.8	6.23	6.90	8,500
46	12	10.4	9.7	4.68	3.97	12,500
47	8	12.8	12.0	5.49	5.40	10,500
48*	7	9.2	8.0	4.64	4.63	9,000
49	7	9.3	9.1	4.96	4.67	8,500

* This dog died of bronchopneumonia on the last day.

tube. The plasma proteins were essentially unaffected by starvation, as had been shown years ago by Kerr, Hurwitz and Whipple.[27] No gross edema developed. Loss of weight occurred in the seven dogs, varying between 2 and 9 per cent of the initial body weight. Table 7 gives a summary of these experiments.

The daily administration of 1,500 cc. of 0.85 per cent sodium chloride solution to a dog of 10 Kg. may seem to be a rather excessive dose. However, dogs can dispose of even larger amounts of salt and water without developing edema. As a matter of fact, one of the probable reasons why McIntosh and Leiter[28] were unable to produce edema by acute plasmapheresis in more than two dogs of a considerable number was the daily administration to these dogs of about 40 Gm. of sodium chloride as a hypertonic solution. This resulted in a negative chloride balance even in dogs in which diarrhea did not develop. Rela-

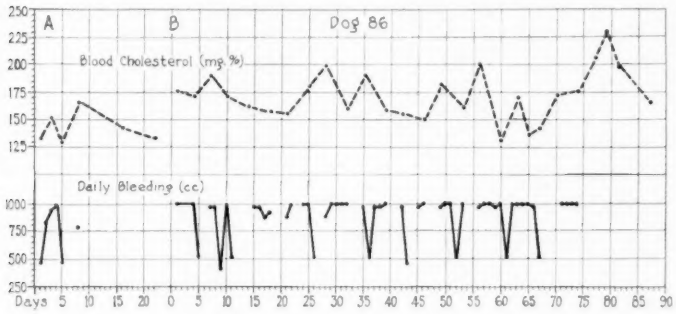

Fig. 8.—In spite of repeated increases in the blood cholesterol, there is no tendency for a persistent hypercholesterolemia in this chronic experiment.

tive to body weight, the dog's kidneys have a remarkable capacity to dispose of chlorides. In the present experiments, as shown in figure 4, the continued administration of saline has little or no influence on the tide of disappearing edema once the plasma proteins have surpassed the critical edema level. At most, a slight hump in the weight curve manifests itself for a day or two.

In nephrotic Bright's disease a low concentration of plasma protein is usually associated with a low albumin-globulin ratio. This has often been attributed to the fact that most of the protein lost in the urine is plasma albumin, but little of it is plasma globulin. In experimental

27. Kerr, H. J.; Hurwitz, J. H., and Whipple, G. H.: Am. J. Physiol. **47**:356, 1918.

28. McIntosh and Leiter: Unpublished observations of experiments during 1925 and 1926.

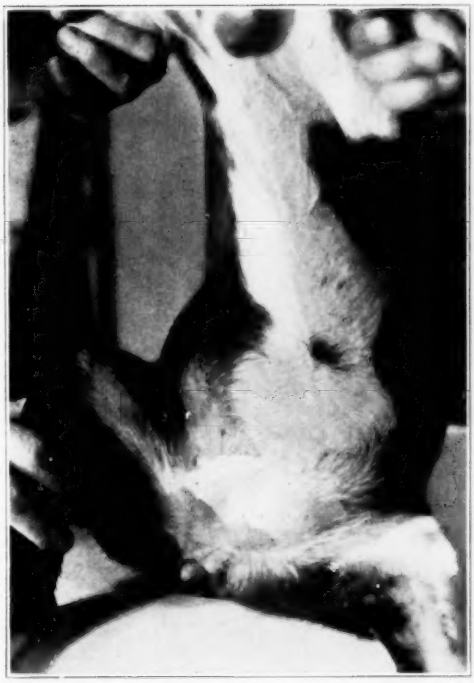

Fig. 9 (dog 10).—Ascites and edema of the abdominal wall, perineum and thighs. (See table 2 and fig. 1.)

Fig. 10 (dog 17).—Ascites and edema of the wall of the lower part of the abdomen, external genitalia and hind limbs. (See table 3 and figs. 4 and 5.)

plasmapheresis no such explanation holds, since the entire plasma with its particular albumin-globulin ratio is discarded. Yet in the longer experiments there is a marked decrease or reversal in the albumin-globulin ratio. This is shown in table 8 and figure 5. Incidentally,

TABLE 8.—*Protocol of Long Experiment Showing the Effects of Depletion of Plasma on the Plasma Albumin and Globulin and the Blood Cholesterol*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Plasma Albumin, Gm. per 100 Cc.	Plasma Globulin, Gm. per 100 Cc.	Blood Cholesterol, Mg. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
86	12/ 2/29	12.2	435	400	0.25	5.28	2.97	2.31	131	1,500	Blood drawn from external jugulars
A	12/ 3/29	12.0	770	850	....	....	....	....	...	1,500	
	12/ 4/29	12.0	870	950	0.19	4.13	2.08	2.05	149	1,500	
	12/ 5/29	12.0	920	825	....	....	....	....	...	1,500	Not eating
	12/ 6/29	11.6	435	450	0.34	2.92	1.88	1.05	128	1,000	500 cc. of milk by stomach tube; no edema
	12/ 7/29	11.7	...	...	....	....	....	....	...	1,000	500 cc. of milk by stomach tube
	12/ 9/29	11.1	745	850	0.16	4.28	1.54	2.74	165	1,500	Eating; jugulars closed
	12/23/29	....	...	...	0.19	4.61	1.80	2.81	133	.....	
B	1/21/30	12.7	895	875	0.22	4.30	2.44	1.86	175	1,500	Cardiac bleeding
	1/22/30	12.7	920	975	....	....	....	....	...	1,500	
	1/23/30	12.5	920	800	....	....	....	....	...	1,500	
	1/24/30	12.8	920	1,000	0.20	2.48	1.19	1.29	172	1,500	Slight edema
	1/25/30	12.9	460	500	....	....	....	....	...	1,000	Unchanged
	1/27/30	12.5	895	1,000	0.22	4.33	1.40	2.93	191	1,500	No edema
	1/28/30	12.6	895	1,000	....	....	....	....	...	1,500	
	1/29/30	12.8	360	500	....	....	....	....	...	1,500	
	1/30/30	12.6	920	1,000	0.38	3.21	1.03	2.18	171	1,500	No edema
	1/31/30	12.8	460	500	....	....	....	....	...	1,500	
	2/ 1/30	12.8	...	...	....	....	....	....	...	1,000	
	2/ 3/30	12.5	...	...	0.23	4.77	1.01	3.76	162	1,500	Good condition
	2/ 4/30	12.5	895	975	....	....	....	....	...	1,500	
	2/ 5/30	12.6	895	1,000	....	....	....	....	...	1,500	
	2/ 6/30	12.8	795	1,000	....	....	....	....	...	1,500	
	2/ 7/29	12.8	845	1,000	0.23	3.62*	0.85	2.77	157	1,000	No edema; convulsions
	2/ 8/30	12.9	...	.....	....	....	....	....	...	1,000	Good condition
	2/10/30	12.6	795	1,000	0.26	4.52*	1.43	3.09	155	1,500	
	2/11/30	12.7	920	950	....	....	....	....	...	1,500	
	2/13/30	12.5	920	1,000	....	....	....	....	...	1,500	
	2/14/30	12.5	920	1,000	0.28	3.80*	1.19	2.61	179	1,500	
	2/15/29	12.5	460	400	....	....	....	....	...	1,000	Slight edema
	2/17/30	12.0	895	1,000	0.17	3.66*	1.38	2.28	199	1,500	
	2/18/30	12.7	920	925	....	....	....	....	...	1,500	Slight edema of thighs
	2/19/30	12.8	920	1,000	....	....	....	....	...	1,500	
	2/20/30	13.0	920	1,000	....	....	....	....	...	1,500	No edema
	2/21/30	13.0	920	1,000	0.20	2.08	1.24	0.84	160	1,500	Slight edema
	2/24/30	12.5	870	1,000	0.19	4.52	1.36	3.16	191	1,500	No edema
	2/25/30	12.7	460	500	....	....	....	....	...	1,500	
	2/26/30	12.8	895	1,000	....	....	....	....	...	1,500	
	2/27/30	13.1	895	1,000	....	....	....	....	...	1,500	
	2/28/30	13.6	920	900	0.14	3.54	1.44	2.10	158	1,500	Edema of thighs and legs
	3/ 1/30	13.3	...	...	....	....	....	....	...	1,000	
	3/ 3/30	12.5	870	925	0.18	3.91	1.75	2.16	155	1,500	No edema; good condition
	3/ 4/30	12.5	410	375	....	....	....	....	...	1,500	
	3/ 6/30	12.0	870	1,000	....	....	....	....	...	1,500	
	3/ 7/30	12.2	920	1,000	0.26	4.04	2.15	1.89	150	1,500	No edema
	3/ 8/30	12.6	...	.....	....	....	....	....	...	1,000	

TABLE 8.—*Protocol of Long Experiment Showing the Effects of Depletion of Plasma on the Plasma Albumin and Globulin and the Blood Cholesterol—Continued*

Dog	Date	Weight, Kg.	Blood Drawn, Cc.	Erythrocytes Returned, Cc.	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Plasma Albumin, Gm. per 100 Cc.	Plasma Globulin, Gm. per 100 Cc.	Blood Cholesterol, Mg. per 100 Cc.	Saline by Stomach Tube, Cc.	Comment
B	3/10/30	12.5	895	1,000	0.13	4.83	1.51	3.32	183	1,500	
	3/11/30	12.7	920	1,000	....	....	....	....	...	1,500	
	3/12/30	12.8	920	950	....	....	....	....	...	1,500	Slight edema of thighs
	3/13/30	12.9	460	500	....	....	....	....	...	1,500	
	3/14/30	12.9	920	1,000	0.22	3.09	1.18	1.91	161	1,500	
	3/15/30	12.9	...	.....	....	....	....	....	...	1,000	
	3/17/30	12.6	895	925	0.26	4.66	1.38	3.28	200	1,500	Convulsion; no edema
	3/18/30	12.7	920	1,000	....	....	....	....	...	1,500	
	3/19/30	12.8	920	1,000	....	....	....	....	...	1,500	Slight edema
	3/20/30	12.9	895	975	....	....	....	....	...	1,500	More edema
	3/21/30	12.9	920	1,000	0.16	3.62	1.33	2.29	130	1,500	
	3/22/30	12.9	460	275	....	....	....	....	...	1,500	Edema increasing
	3/23/30	13.4	920	825	....	....	....	....	...	1,500	
	3/24/30	13.8	920	1,000	0.13	2.97	1.51	1.46	171	1,500	
	2/25/30	14.0	920	1,000	....	....	....	....	...	1,500	Marked edema of external genitalia, legs, etc.
	3/26/30	13.5	920	1,000	0.15	2.42*	1.06	1.36	134	1,500	
	3/27/30	14.6	895	825	....	....	....	....	...	2,500	Ascites; edema of walls of abdomen and chest; hemolytic icterus
	3/28/30	14.0	460	425	0.20	3.23	1.02	2.21	142	.....	Edema unchanged; icterus decreasing
	3/29/30	13.5	...	...	....	....	....	....	...	1,000	Edema decreasing rapidly
	3/31/30	10.8	...	...	0.19	4.79	1.80	2.99	172	.....	No edema; good condition
	4/ 1/30	11.0	920	950	....	....	....	....	...	1,500	Double rations from this date
	4/ 2/30	11.2	920	1,000	....	....	....	....	...	1,500	
	4/ 3/30	11.6	920	1,000	....	....	....	....	...	1,500	Edema of thighs
	4/ 4/30	11.8	920	1,000	0.16	4.28	1.49	2.79	175	1,500	
	4/ 5/30	12.6	...	.....	....	....	....	....	...	1,000	Edema more marked
	4/ 7/30	11.7	...	.....	0.21	5.93	1.41	4.52	206	1,500	No edema
	4/ 8/30	11.8	...	.....	....	....	....	....	...	.....	
	4/ 9/30	12.0	...	.....	0.15	6.13	1.61	4.52	230	1,500	Acute arthritis of both ankles
	4/10/30	12.3	...	.....	....	....	....	....	...	1,000	
	4/11/30	12.4	...	.....	0.11	5.33	1.91	3.42	195	.....	
	4/12/30	12.4	...	.....	....	....	....	....	...	.....	
	4/14/30	11.6	...	.....	0.34	7.56	2.03	5.53	216	.....	Condition good
	4/15/30	11.8	...	.....	....	....	....	....	...	.....	Ankles normal
	4/16/30	12.0	...	.....	....	....	....	....	...	.....	
	4/17/30	12.2	...	.....	0.16	7.24	1.71	5.53	165	.....	
	4/18/30	12.4	...	.....	....	....	....	....	...	.....	Eating well
	4/19/30	12.5	...	.....	....	....	....	....	...	.....	Sudden dyspnea and death from acute pulmonary edema; no ascites

* Sample of blood drawn after first dose of saline.

the immediate increase in the plasma proteins, subsequent to discontinuance of plasmapheresis, is largely due to a rise in the globulin fraction.[29] The parallelism between the total protein and the globulin curves is often striking. In the experiments of two weeks' duration or less no such regularity is ordinarily evident. It takes considerable time for plasma albumin to regenerate, although there is much variation

29. Leiter, L.: Proc. Soc. Exper. Biol. & Med. **27**:1002, 1930.

in individual dogs. The edema level of the concentration of plasma protein is determined not simply by the amount of plasma albumin, but by the resultant of the colloidal osmotic pressure due to both the albumin and the globulin fractions. Increase in globulin, if sufficiently developed, may counteract the effects of a low or even decreasing plasma albumin.

The rapid restoration of plasma globulin is not entirely dependent on a good state of nutrition of the animal. In dog 90, for example, the emaciation manifested by a fall in body weight from 12.6 to 8.9 Kg. in the course of thirty days was not sufficient to prevent the typical sharp rise in the plasma globulin curve at the end of this experimental period. No dog was observed in which a significant increase in plasma

Fig. 11 (dog 34).—Dog with massive rolls of edematous anterior thoracic and abdominal walls, following acute hydremic plethora during a period of hypoproteinemia. (See table 4 and fig. 6.)

globulin did not occur within from twenty-four to forty-eight hours after bleeding was interrupted.

When cholesterol of the blood was used as an indicator for the blood lipoids, a curious series of results was obtained.[29] In the short experiments (fig. 6) no consistent trends could be made out. However, with prolonged depletion of plasma there appeared a more or less definite cycle of hypocholesterolemia during active bleeding followed by temporary hypercholesterolemia in the intervals free from plasmapheresis. Similar conditions are evident in some of the protocols of Barker and Kirk,[17] although these authors have drawn rather different conclusions from their results. Figures 7 and 8 and table 8 indicate the cycles described. While it is possible that the lower values for

blood cholesterol during vigorous production of depletion of plasma may depend partly on a decreased concentration of erythrocytes, this is not probable in view of the fact that the reinjected erythrocyte-saline mixture was practically always made up to a normal relative volume of red corpuscles by the addition of donor's erythrocytes. However, this phase of the problem will bear repetition, plasma cholesterol instead of the whole blood cholesterol being used as the basis for comparison.

The anatomic changes observed during these experiments were of considerable variety. Some were due directly to the procedure employed; others were incidental. The changes in the heart and kidneys were the most significant. Such lesions as bronchopneumonia, infarcts or

Fig. 12.—Typical subcutaneous and intermuscular edema in dog 32. Low power view.

abscesses in the lungs and spleen of some animals and traumatic hemothorax require no comment. If the dog died during a period of active edema, ascites, pleural effusions and pulmonary edema were common accompaniments of subcutaneous anasarca. Occasionally hemolytic icterus developed from the rapid destruction of foreign blood.

In the heart, a great many traumatic lesions were encountered. These were largely the result of the needle punctures. There were linear areas of hemorrhage, necrosis, leukocytic infiltration and various stages of repair by granulation in the myocardium of the right and left ventricles and often in the septum. In spite of a great number of puncture wounds, the pericardial sac was never completely obliterated, nor was there any gross suppuration except in two dogs, one of which

(dog 25) had a severe infection of the respiratory tract. Usually the parietal pericardium was thickened and adherent over the anterior aspect of the right ventricle, in which most of the punctures were made. Localized deposits of fibrin were also seen. The endocardium was frequently lacerated, and mural thrombi were common. In a number of dogs considerable damage was done to the tricuspid, aortic and mitral valves, with resulting vegetations of a friable character that gave rise to emboli. Fibrous thickenings and fusion of valves were seen in some dogs, involving particularly the tricuspid and mitral valves. Puncture of a coronary vein or artery on the surface of the heart invariably led to death within a matter of minutes and could be diagnosed from the clinical symptoms after a few experiences. Only occasionally was a mass of fibrin found in the pericardium, indicating a previous nonfatal hemopericardium. Dogs 17, 34, 86 and 89, which had periods varying between fifteen and fifty-four days of actual bleeding, involving a minimum of from 52 to 198 cardiac punctures, showed no gross blood or residual coagulum in the pericardial sac at autopsy.

The kidneys were the seat of several types of pathologic processes. In the first place, gross infarcts were common because of the frequency of endocardial thrombosis. These infarcts were bland or frankly infected. In dogs 71, 73, 86, 89 and 90, in which time for organization of the infarcts was available, there was extensive calcification of necrotic tubules and glomeruli, along with the usual interstitial fibrosis and some round cell infiltration. In these areas, also, exquisite masses of doubly refractile lipoid droplets could be seen in the casts and atrophying tubular epithelium. Another lesion was localized cortical suppuration, in the form of acute glomerulitis and periglomerulitis, of embolic origin, often associated with linear leukocytic infiltration and necrosis extending to the lower cortex or ending in abscesses in various levels of the medulla. These changes in the kidney, like the infarcts, were easy to understand. Another type of process, however, gave more trouble. This consisted of multiple irregular or linear foci of large mononuclear cells ("plasma cells") with a smaller number of lymphocytes and polymorphonuclears, found about the interlobular arteries and veins or around the glomeruli and between the tubules, often extending through the entire cortex and obviously leading to compression atrophy of the tubules coursing through these cellular masses. In some kidneys the infiltrates were near an infarcted or suppurative area. In others, however, they constituted the only lesions. There were clearcut transitions between such mononuclear, perivascular infiltrates in the cortex and definite linear or wedge-shaped scars in which tubules had atrophied or disappeared while glomeruli were still preserved but with concentric

layers of fibrous connective tissue about the capsules and some shrinkage of the entire tuft. The vessels in these scarred areas were not significantly altered. Such scars, when numerous, may give the superficial impression of a contracted kidney because of the pits and depressions on the surface. A large amount of normal parenchyma, however, is usually present between the narrow scars. It is in this group of renal lesions, probably, that one must place the changes described by Barker and Kirk [17] and Barker,[30] who attributed them directly to a humoral effect of the altered blood plasma and speculated on their possible relation to human contracted kidneys. These lesions will be discussed in more detail later on.

Fatty changes in the form of irregular accumulation of large droplets in cells of ascending limbs of Henle's loops and the terminal straight portions of the proximal convoluted tubules in the lower part of the cortex were observed rather frequently. Practically none of this fat was doubly refractile. Fatty casts and calcified casts or epithelium in various levels of the medulla were inconstant observations. Careful search for doubly refractile lipoid droplets revealed a few typical fluid crystals in many of the convoluted tubules in the middle of the cortex. These droplets were usually seen at the apex of the cells or actually in the narrow lumen. Like the ordinary fat in the inner cortex, these isolated droplets probably represent a physiologic condition. No changes were observed in the convoluted tubules comparable to the typical morphology of chronic nephrosis in man.

COMMENT

The low protein content of the edema fluid produced by depletion of plasma establishes it as a nephrotic type of edema analogous to the transudates in nephrotic Bright's disease, in war edema and in other nutritional dropsies. The correlation between low serum or plasma proteins and the presence of nephrotic edema in man is so well established [31] that it requires no further review here. In rats a nutritional edema, probably nephrotic, has been repeatedly produced by diets low in protein only [32] and correlated with reduction in serum proteins.[33] Harden and Zilva [34] commented on a nutritional edema observed in a monkey. Through the cooperation of Dr. I. S. Falk, formerly in the Department of Bacteriology of the University of Chicago, the data

30. Barker (footnote 18, first reference).

31. Leiter, L.: Nephrosis, Medicine **10**:135 (May) 1931.

32. Kohman, E. A.: Am. J. Physiol. **51**:185 and 378, 1920. Frisch, R. A.; Mendel, L. B., and Peters, J. P.: J. Biol. Chem. **84**:167, 1929.

33. Frisch, Mendel and Peters (footnote 32, second reference).

34. Harden, A., and Zilva, S. S.: Lancet **2**:780, 1919.

shown in table 9 have been obtained and represent the first published analyses on a nephrotic type of edema in the monkey. The exact origin of the nutritional disturbance is unknown. Histologic examination of the kidneys eight weeks after the original edema had subsided revealed no pathologic changes. Figure 13 shows the edema of the eyelids in this monkey.

The edema produced in dogs has been shown to be definitely related to a decrease in the plasma proteins to approximately half of the normal

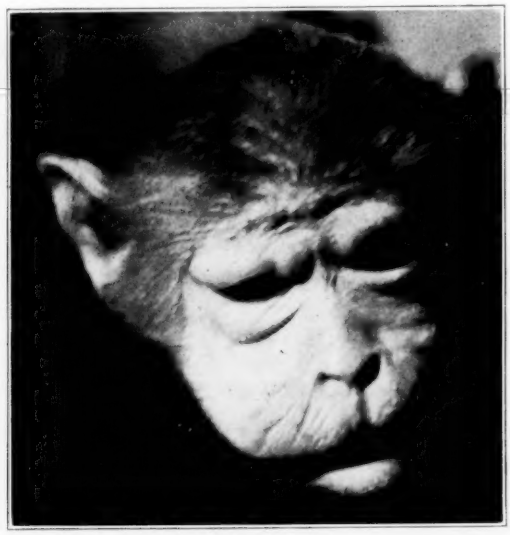

Fig. 13.—Edema of the eyelids in a monkey that also had edema of the abdominal wall and perineum. (See table 9.)

value. A slight increase in the plasma proteins above this level has been regularly associated with decrease or disappearance of edema. The rapidity with which this change occurs speaks strongly in favor of a simple disturbance in the equilibrium of mechanical forces determining the interchange of fluids between the capillaries and the tissue spaces. No nutritional disturbance of the walls of the capillaries, as might be postulated to be the consequence of the hydremia, no cardiac or renal insufficiency and no change in the tissue colloids can reasonably be assumed to afford a better explanation of this rapid change in water balance. Furthermore, since the capillaries are normally freely permeable to salt and water in both directions,[35] any theory involving

35. Krogh, August: Anatomie und Physiologie der Capillären, Berlin, Julius Springer, 1924.

altered permeability to salt and water as a result of some toxic or functional change in the wall of the capillary has little significance. If, on the other hand, the true concept of permeability of the capillaries is understood, that is, the permeability to the plasma proteins, there is no evidence that the experimental edema described is related to increased permeability. There is almost no protein in the edema fluid. This observation is in striking contrast to practically all of the experimental edemas previously reported. Thus, the ascitic fluid in uranium

TABLE 9.—*Protocol on a Monkey with Nephrotic Edema of "Spontaneous" Development*

Date	Weight, Kg.	Total Plasma Nitrogen, Gm. per Liter	Plasma Nonprotein Nitrogen, Gm. per Liter	Plasma Proteins, Gm. per 100 Cc.	Protein in Edema Fluid, Gm. per 100 Cc.	Comment
1/12/29	.....	5.90	0.53	3.36*	....	Edema of the abdominal wall, perineum and thighs; diarrhea
1/16/29	3.475	....	....	....	....	Edema unchanged; blood creatinine, 1.16 mg.
1/17/29	3.350	....	....	....	....	Blood urea nitrogen, 14.3 mg.; trace of albumin in the urine
1/19/29	3.475	....	....	....	....	Edema of the scalp, eyelids and cheeks; eating ravenously
1/21/29	3.375	6.29	0.20	3.80	....	No edema of the face; persistent edema of the perineum and lower part of abdomen
1/23/29	3.400	....	....	....	0.39	About 10 per cent blood in this sample of edema fluid
1/24/29	3.375	5.38	0.21	3.23	0.12†	
1/25/29	3.150	....	....	....	....	Edema decreasing
1/29/29	3.150	....	....	....	....	Morning edema of the face; photographed
2/ 4/29	3.000	....	....	....	....	No diarrhea; edema decreasing
2/ 9/29	2.900	....	....	....	....	Slight edema of abdominal wall
2/11/29	2.800	....	....	....	....	No edema
2/19/29	2.900	7.56	0.39	4.48	....	No edema; good condition
2/23/29	2.900	....	....	....	....	Returned to Dr. Falk's laboratory
4/11/29	.....	4.69	0.46	2.66	....	Died in emaciated state with moderate peripheral edema and some ascites; no tuberculosis; no nephritis

* The percentage of plasma protein in two normal monkeys was 6.28 and 6.84.
† The protein content of ascitic fluid from a monkey with tuberculous peritonitis was 2.85 per cent. The percentage of plasma protein was 4.93.

edema coagulates spontaneously and has a protein content ranging between 1 and 3 per cent.[36] This indicates a close relationship to the inflammatory edemas produced by such obvious vascular poisons as mustard oil, paraphenylenediamine and dionin, in which the exudate is often whole plasma.[37] Similarly, the edema recently produced by subcutaneous administration of Ringer's solution to bilaterally nephrec-

36. Garnier, M.; Schulmann, E., and Marek, J.: Compt. rend. Soc. de biol. **99**:269, 1928. Nau, A.: Compt. rend. Soc. de biol. **99**:869, 1928. Govaerts, P.: Bull. Acad. roy. de méd. de Belgique **9**:33, 1928. Georgopulos (footnote 8).

37. Tainter, M. L., and Hanzlik, P.: J. Pharmacol. & Exper. Therap. **24**:179, 1924.

tomized dogs also has a protein content between 1 and 3 per cent.[38] It is fair, therefore, to state that none of these experimental edemas is pathogenetically related to nephrotic edema. Conversely, clinical and experimental nephrotic edemas are primarily not problems of permeability of the capillaries, but rather disturbances in the normal equilibrium between hydrostatic and protein osmotic forces. The experimental nephrotic edema in dogs thus represents the first direct evidence in favor of the Starling-Epstein-Krogh theory of filtration edemas. In dogs, the edema is uncomplicated by factors of permeability. In man, especially in early nephrotic glomerulonephritis, the capillaries may remain partially injured shortly after the acute stage of the disease, so that edema fluid obtained during this period may contain protein intermediate in value between nephrotic and acute nephritic edemas. On the other hand, in hunger edema there is no evidence of increased permeability of the capillaries to protein.

The edema in dogs differs from the purely nutritional edemas of man and experimental animals in being a much more acute process. While in the rat it takes about two months of feeding on a low protein diet before edema manifests itself, plasmapheresis in the dog is only a matter of days. The period of undernutrition requisite to produce edema in man is still an unknown quantity. On the other hand, the direct loss of plasma proteins in the dog is a more violent disturbance than the gradual depression of the building of plasma proteins because of the inadequate supply of their elements in undernutrition. The intensity of the process in the dog, therefore, more than outweighs the time factor in ordinary nutritional edemas. A clinical parallel is seen in those cases of acute glomerulonephritis in which a marked albuminuria persists from the start, so that within a relatively short time the plasma proteins become reduced to the level of the edema. It is perhaps reasonable to assume that in all these clinical and experimental states one is dealing with different degrees of the same underlying process.

The rôle of salt and water in the edema of dogs would seem to be relatively simple. The administered saline is not the cause of edema any more than salt and water are the actual cause of most clinical edemas. However, no edema is possible without an endogenous or exogenous supply of the chief constituents of edema fluid. Hence, all intercellular edemas are to this degree dependent on a proper supply of salt and water. Therefore, it is not at all remarkable that patients may lose some of their edema without any significant increase in the plasma proteins, provided they are kept on a low intake of salt and

38. Farmer, C. J.; Barry, F. S.; Reed, A., and Ivy, A. C.: Proc. Inst. Med., Chicago, 1930, vol. 8.

fluid or are given diuretics that somehow produce a negative salt and water balance. Govaerts[39] made this point clear. This situation has been repeatedly overlooked by authors who are unwilling to accept the important position of the plasma proteins in certain clinical edemas. In the dog, the same amount of salt and water that produced edema when the level of plasma protein was below 3 per cent fails to have any effect when the plasma proteins reach 3.5 or 4 per cent. Although the actual experiment has not been carried out, there is little doubt but that it should be possible to keep a dog entirely free from edema on a rigidly salt-poor and low fluid diet even with the plasma proteins reduced to the level for edema. In patients or animals with the plasma proteins just at the critical level, the effect of the intake of sodium chloride and water on the presence of edema will naturally be more striking because under these conditions the body responds quickly one way or the other. This is well illustrated in cachectic states, in hunger edema and in various other nutritional edemas in which the plasma proteins may be only moderately reduced. Rest in bed and restriction of salt and fluid are often excellent diuretics in such patients.

The rapidity with which the dog can regenerate plasma proteins even during emaciation is a surprising phenomenon, but again illustrates the large factor of safety involved in all vegetative functions. How much the ordinary fractionation of the plasma proteins really means concerning the actual nature of the proteins involved is unknown, yet the results have been quite consistent in indicating a more rapid formation of the globulin fraction than of the albumin fraction. It must be admitted, however, that the effect of infection was not controlled in our dogs. It is interesting at this point to consider the work of Bruckman, D'Esopo and Peters,[40] who observed that the serum albumin was essentially the only fraction reduced in the moderate hypoproteinemia of malnutrition from various causes. Improved nutrition was associated with an increase in the serum albumin. Edema occurred in the patients with low serum proteins and could be fairly well correlated with low serum albumin.[41] The absolute quantity of plasma proteins lost daily by dogs undergoing plasmapheresis has not been determined, but it is safe to estimate that it corresponds to a severe albuminuria in man. Both the patient with nephrotic Bright's disease and the dog may maintain a fairly constant level of plasma proteins while losing 10 Gm. or more of protein a day. They are both regenerating proteins

39. Govaerts, P., and Cordier, R.: Bull. Acad. roy. de méd. de Belgique **8**:510, 1928.

40. Bruckman, F. S.; D'Esopo, L. M., and Peters, J. P.: J. Clin. Investigation **8**:577, 1930.

41. Bruckman, F. S., and Peters, J. P.: J. Clin. Investigation **8**:591, 1930.

quite actively. One wonders, therefore, what the patient could do if he were really able to procure and assimilate large amounts of food protein. Although the high protein therapy of nephrotic Bright's disease has been suggested and championed by Epstein [42] for many years, no systematically controlled study of the effects of varying amounts of dietary protein in this disease has been available until the recent work of Peters and Bulger [43] and of Berglund and his associates.[44]

In recent years there has been a considerable tendency to ascribe practically all of the demonstrable biochemical and morphologic changes in the nephrotic syndrome to the loss of plasma protein in the urine. It does not matter for this point of view whether the protein is or is not biologically foreign to the organism. The important fact is its escape from the plasma. The relatively constant hypercholesterolemia (lipoidemia) in the active stages of the nephrotic syndrome naturally suggested a systematic study of the cholesterol of the blood in the dogs undergoing plasmapheresis. Barker and Kirk [17] reported increases in blood cholesterol up to 254 mg. per hundred cubic centimeters, and correlated this with the well established data on patients with nephrosis. An examination of their published protocols actually leads to the conclusion that active depletion of plasma results in a definite hypocholesterolemia with a temporary rebound to higher values when bleeding has been interrupted. Our results, obtained on a more representative series of dogs, also seem to indicate that plasmapheresis and edema are associated with a decrease in the blood cholesterol. This is a picture exactly the opposite of the situation in the nephrotic syndrome in man. In the latter, the blood cholesterol usually remains elevated for long periods during the albuminuric, edematous stages of the disease, falling toward normal when the cardinal symptoms clear up. Granting this difference between the experimental nephrotic edema and the clinical nephrotic syndrome, it is obviously incorrect to describe the experimental condition as "nephrosis" and to attempt to force it into more or less complete alinement with nephrotic Bright's disease, as Barker and Kirk [17] have done. Why it is that nephrotic Bright's disease is characterized by an increase in blood cholesterol while hunger edema, with essentially the same type of transudates, usually is associated with a decrease in blood cholesterol, remains a difficult puzzle. The gaps in the knowledge of lipoid metabolism are too many to allow anything but a guess as to the reasons for the curves for blood cholesterol observed in dogs.

42. Epstein, A. A.: Arch. f. Verdauungskr. **44**:31, 1928; footnote 14.

43. Peters, J. P., and Bulger, H. A.: Relation of Albuminuria to Protein Requirement in Nephritis, Arch. Int. Med. **37**:153 (Feb.) 1926.

44. Berglund, H.: Symposium on the Kidney, Minneapolis, July, 1930.

One may conclude from the foregoing discussion that experimental nephrotic edema is far from being synonymous with the nephrotic syndrome in man. A symptom, edema, has been produced, and not much else. There still remains to be considered the so-called renal lesion of plasmapheresis. Barker [30] stated that "a secondary contracted kidney may well follow a long standing low proteinemia as a result of tubular atrophy and scar tissue replacement" (page 609). Earlier, Barker and Kirk [17] were not quite so definite in their conclusions, although they wrote that "the experimental results tend to support the theory that the continual loss of albumin in the urine is sufficient alone to produce renal pathologic changes" (page 345). If this point of view could be established beyond a reasonable doubt, it would represent an important contribution toward the explanation of the contracted kidneys the etiology of which is not an obvious chronic glomerulonephritis or arteriolar sclerosis. As a matter of fact such kidneys, excluding, of course, hydronephrotic and pyelonephritic types, are extremely rare. Furthermore, there are so few cases of proved nephrotic contracted kidneys,[31] that is, contraction following on a genuine or pure nephrosis, that most pathologists have never seen any. Barker's [30] suggestion, therefore, that his experimental renal lesions might explain "why most so-called nephrosis cases that escape intercurrent infections, die of uremia and at post mortem show scarred and contracted kidneys" is irrelevant, since most cases of so-called nephrosis, as has been so clearly demonstrated by Bell,[45] are obvious forms of glomerulonephritis, easily recognized by experienced pathologists, if not by clinicians.

Apart from this question, the more important one arises as to the actual nature of these renal lesions in dogs and their relation to plasmapheresis. So far, Barker's evidence is based on observations made on a few dogs. Even a superficial familiarity with "spontaneous" nephropathy in dogs immediately leads one to question the pathogenesis of these renal changes. Many authors have described "spontaneous" lesions in dogs' kidneys as such or have mistaken them for something they wanted to produce. Long experiments are particularly dangerous, for even a preliminary biopsy, proving the absence of certain lesions, does not rule out the possibility of spontaneous development under laboratory conditions later. This has been well demonstrated in the excellent work of Bell and Hartzell.[46] The papers of Barker and Kirk present no satisfactory controls.

It is perhaps fortunate that in the present investigation many dogs lived only a short period. Therefore a large series of kidneys was

45. Bell, E. T.: Am. J. Path. **5**:587, 1929.

46. Bell, E. T., and Hartzell, T. B.: Spontaneous Nephritis in Rabbits and Its Relation to Chronic Nephritis in Man, J. Infect. Dis. **24**:628 (June) 1919.

studied. Some control experiments were carried out. This gives an opportunity to compare the renal lesions in various ways, that is, in relation to the duration of plasmapheresis, to the presence or absence of gross infection in the kidneys and to various control procedures. Renal changes more or less comparable to the advanced stages described by Barker and Kirk were found in two dogs; dog 85, which had been bled only three days, was discarded and died of distemper thirty-two days later; dog 16B died in a state of shock on the second day of a control experiment involving the reinjection of his own citrated whole blood. It is fair to conclude that the experimental procedure could not have produced the extreme fibrosis in the kidneys of these two dogs in such a short time. On the other hand, in dogs 17, 34, 35, 69, 71, 86, 89 and 90, on which vigorous plasmapheresis was performed eighteen, twenty-four, twenty-nine, twenty-four, fifteen, sixty, eighteen and thirty-seven days, respectively, during a total experimental period of thirty-one, thirty-six, forty-seven, thirty-one, twenty-eight, one hundred and thirty-nine, thirty-four, and ninety-eight days, respectively, the renal lesions, apart from infarcts, abscesses and other changes expected with the method used for bleeding, consisted almost entirely of varying degrees of infiltration with mononuclear cells. In fact, in dogs 17, 35 and 69 there were no infiltrates. These perivascular and intertubular collections of "plasma cells," lymphocytes and occasionally polymorphonuclears were also found in the renal cortex of dogs which had died after very short periods of plasmapheresis. Thus dogs 14, 15, 32, 33 and 72 with experimental life periods of only five, six, five, eight and three days, respectively, showed from slight to marked mononuclear infiltration. Are these the result of, or are they related to, hypoproteinemia? One answer to this question is the observation in dog 39C of many linear and wedge-shaped areas of mononuclear infiltration with corresponding atrophy of the tubules. Dog 39C was a control animal transfused for twenty of twenty-nine days with from 200 to 400 cc. of other dogs' blood daily. No depletion of plasma was carried out, and the plasma proteins varied between 4.77 and 5.28 per cent during the last eleven days of the dog's life. Another answer is the relatively extensive literature [47] on more or less identical lesions reported from many parts of the world in connection with work on rabbits, dogs, cattle and cats. This is not the place to discuss the etiologies of such lesions in the kidney. It should be noted, however, that in our experiments vegetative endocarditis was frequent and asepsis was not employed, while Barker and Kirk used careful aseptic technic. The results are difficult to distinguish in the two series. What is most significant, however, is the

47. Leiter, L.: Experimental Chronic Glomerulonephritis, Arch. Int. Med. **33**: 611 (May) 1924; Data to be published.

complete lack of relation between these acute and chronic truly interstitial nephritides in animals and chronic nephritis in man. This has been pointed out before,[48] but apparently still needs emphasis in view of the recent confusion. The lack of correlation between low plasma proteins and these renal lesions is self-evident from the few control experiments reported.

It seems from this discussion that the renal lesions following plasmapheresis may have nothing to do with plasmapheresis except so far as dogs are followed over a long enough period to allow for the full development of "spontaneous" lesions or for changes possibly due to the repeated intravenous injection of particulate matter—erythrocytes, more or less damaged by centrifugation, saline solutions and what not. One could not, of course, consider these lesions as a "nephrosis," if one understands by "nephrosis" that form of renal pathology regularly associated with the nephrotic syndrome in man. The fatty changes described in dogs' kidneys are so nonspecific as to be almost physiologic.[49] Deposition of doubly refractile lipoid (cholesterol ester) cannot be found in abnormal amounts in dogs' kidneys, except in calcifying infarcted areas where they are readily demonstrable in masses. This is true in both the long and the short experiments. Here, again, is a striking contrast between the kidneys of dogs with experimental nephrotic edema and the kidneys of patients with "nephrosis" or whatever else it may be called. Experimental edema and "nephrosis" are not synonymous by any means.

SUMMARY AND CONCLUSIONS

Edema has regularly been produced in dogs by adequate plasmapheresis and the resulting reduction in the plasma proteins. The critical level of edema of the plasma proteins is about 3 per cent. Interruption of depletion of plasma is followed by an immediate increase in the plasma proteins and an equally rapid disappearance of edema by diuresis. The disturbance in water balance seems to be largely a temporary upset in the normal equilibrium between the capillary hydrostatic or filtration forces and the plasma protein osmotic or reabsorption forces. It is not primarily dependent on cardiac, renal or capillary permeability factors.

This experimental edema is nephrotic, as demonstrated, for the first time, by actual quantitative analysis of the protein content of the transudates. The low protein content lies exactly within the range

48. Bell, E. T., and Hartzell, T. B.: Effect of Foreign Protein on the Kidney, J. Infect. Dis. **24**:618 (June) 1919. Bloomfield, A. L.: Bull. Johns Hopkins Hosp. **30**:121, 1919. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: Am. J. Path. **1**:247, 1925.

49. Henschen, F.: Acta med. Scandinav. **53**:774, 1921.

of clinical nephrotic edema fluids and is essentially of the same order of magnitude as the concentration of protein in normal cerebrospinal fluid and of other ultrafiltrates of the plasma.

The study of the regeneration of the plasma proteins in the depleted dogs has confirmed the earlier work of Whipple and his associates, which showed that globulin was reformed faster than albumin.

The blood cholesterol tended to decrease during vigorous plasmapheresis. With cessation of bleeding, the cholesterol usually rose, occasionally to values considerably above normal. This was, however, only a temporary phenomenon and was in no way suggestive of the hypercholesterolemia of the nephrotic syndrome in man.

Histologic study of the kidneys in these experiments has failed to produce any clearcut evidence to support the contention of Barker and Kirk that hypoproteinemia leads to contracted kidneys. In fact, the control experiments and other facts reported seem to lead directly to the conclusion that Barker and Kirk have erred in two directions: First, they have overlooked the possibility and the frequency of "spontaneous" contracted kidneys in dogs. Second, they have apparently failed to realize the lack of essential resemblance between the so-called renal lesions of plasmapheresis and the histologic changes in the kidneys of patients with so-called nephrosis. In view of this confusion, it becomes evident that experimental nephrotic edema in dogs is not quite the same thing as "experimental nephrosis." Experimental nephrosis has not yet been produced.

The rôle of starvation, of the administration of salt and water by stomach tube and of certain other factors in the experimental procedure has been controlled to a certain degree.

The relation between experimental nephrotic edema and other experimental and clinical edemas has been discussed.

In collaboration with Dr. I. S. Falk, we have presented the first published data on the protein content of the plasma and of the edema fluid in a "spontaneous" nephrotic edema occurring in a monkey.

IS POLIOMYELITIS A DISEASE OF THE LYMPHATIC SYSTEM?*

MONTROSE T. BURROWS, M.D.
PASADENA, CALIF.

It seems important to point out at this time that acute anterior poliomyelitis (infantile paralysis) may not be primarily a disease of the central nervous system, but that it may be more correctly classed with the diseases of the lymphatic systems of the body. The change that is probably common to all cases of this disease is an enlargement or a hyperplasia of some or all of the lymphatic structures of the organism. Those parts most frequently attacked are Peyer's patches and the solitary follicles of the intestines, the mesenteric lymph glands, the retroperitoneal glands, the peribronchial glands, the tonsils, the lymph glands of the neck, the axillae and the groin. In the later stages of any attack one may often make the diagnosis of the existence of the disease through noting a slight but definite enlargement of one or both of the epitrochlear glands.

While the gross enlargement of the peripheral lymphatic glands is often not marked, it is always present to some degree and is one of the necessary diagnostic signs in the mild and abortive cases. This enlargement of the lymphatic tissues is one of the striking peculiarities of all of the cases that come to autopsy. Not only are those lymphatic structures already mentioned involved, but one may also see hyperplasia both grossly and microscopically in the thymus and the malpighian corpuscles of the spleen.

THE LYMPHATICS OF THE CENTRAL NERVOUS SYSTEM

In the central nervous system, the lymphatics of the body are represented by spaces which surround the blood vessels. These spaces are known as the perivascular spaces. They empty into the subarachnoid space and in turn are thus connected with the ventricles of the brain. The pacchionian granulations are structures along the sides of the veins through which substances are filtered probably also from the subarachnoid space into the veins and the general circulation. These perivascular spaces, like the subarachnoid space, are lined by a single layer of endothelial cells. The pacchionian granulations are areas in which this lining endothelium pouches into the walls of the veins.

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It is these perivascular spaces and the subarachnoid space that become involved in an attack of true poliomyelitis. There is no evidence to show that the nerve tissue is attacked directly. Apparently it suffers only secondarily from the changes that occur primarily in these spaces and in the meninges.

INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM (UNCOMMON IN ANY EPIDEMIC)

While there was a time when it was thought that poliomyelitis was a disease primarily of the central nervous system, it is now known that this is not true. The great mass of cases of this disease never show any involvement of the central nervous system. The symptoms designated by the name poliomyelitis are evidently only complications which may occur in a widespread infectious disease the pathology of which has not been clearly presented, and which in its eventual solution will not be found to be strikingly different in its manner of spread from many of the more common infections that occur in epidemics. While some authors have thought that as many as 25 per cent of the cases in an epidemic show no nervous symptoms, others have considered that such mild cases represent as many as from 65 to 75 per cent of all cases of this disease (Parkes and Neal [1]). As knowledge of this disease has increased, however, it has become evident that even the latter figures are probably too low.

Before Wickman's [2] interesting study of an epidemic in Sweden and other studies made in other places, about 1911, the relation between these milder forms of the disease and those in which paralysis occurs was not generally recognized. The earlier work of Caverly, 1894,[3] had made no impression. It was only after Flexner and Lewis [4] and others had shown it to be a true infectious disease, the virus of which passes readily through a Berkefeld filter, that the manner of its transmission became a problem of general interest. The cases of paralysis or true poliomyelitis were generally widely separated, and it was natural to suspect even then that the virus was carried in some peculiar manner by some animal, insect or other agent. It was difficult to conceive that the

1. Parkes, W. H., and Neal, Josephine B.: Poliomyelitis: A Brief Resume of the Epidemiology, Symptomatology and Treatment, Southwestern Med. **11**:523 (Dec.) 1927.

2. Wickman, Ivar: Acute Poliomyelitis (Heine-Medin's Disease), translated by W. J. M. A. Maloney, monograph 16, New York, J. Nerv. & Ment. Dis. Publ. Co., 1913.

3. Caverly, C. S.: History of an Epidemic of Acute Nervous Disease of Unusual Type, New York M. Rec. **46**:673 (Dec.) 1894.

4. Flexner, S., and Lewis, P. A.: The Transmission of Epidemic Poliomyelitis to Monkeys, J. A. M. A. **53**:1913 (Dec. 4) 1909.

paralysis is, as Amoss [5] termed it, only a "complication" and often a rare complication in a widespread infectious disease which, without involvement of the central nervous system, is so mild that it never leads to death and is often difficult to distinguish from other mild forms of gastro-intestinal disturbance. Since 1913 it has become more and more apparent, however, that this is true; but even now there is a tendency to class this disease as one primarily of the central nervous system rather than in the light of its true pathology. Attention is being centered too much on the rare cases in which paralysis occurs, rather than on the hundreds of mild cases that may be wholly responsible for the spread of the infection. Too many deductions are being drawn from studies on monkeys in which the virus has been introduced directly into the brain or some site chosen by the operator, rather than from the clinical cases in which the natural routes of infection and spread of the disease can be followed more accurately.

MATERIAL

While the study of poliomyelitis and similar infectious diseases is quite removed from my chief endeavors in medicine at this time, it was my good fortune to have played an active part in the attempt to combat and study this disease in the epidemic that existed in the city of Baltimore in the summer of 1916. It was possible at that time not only to study the clinical aspects of the disease, its epidemiology, its treatment and its bacteriology, but also to obtain excellent pathologic material. By police order, the city granted an autopsy on every fatal case. As acting resident pathologist of Johns Hopkins Hospital, I performed most of these autopsies myself. In each instance, a complete autopsy was performed, and all the organs were studied not only for the gross, but for the microscopic, changes.

These autopsies, together with a rather extensive clinical investigation of the disease, have given an opportunity to correlate more carefully the clinical course of the disease with the pathology, and to obtain a point of view somewhat different from that held by those who look at the disease largely as a primary poliomyelitis rather than a systemic disease that may have a central nervous complication.

I have already reviewed briefly the pathology of this disease in an attempt to separate it from epidemic encephalitis [6] and to find the differential pathologic peculiarities of the latter affliction. The correlations of the pathologic symptoms and signs with the clinical symptoms have not been published. It seems important to present them now.

5. Amoss, H. L.: Poliomyelitis, South. M. J. **23**:18 (Jan.) 1930.

6. Burrows, M. T.: Neuritis of the Cranial Nerves in Lethargic Encephalitis and the Differential Anatomic Diagnosis Between It and Acute Poliomyelitis, Arch. Int. Med. **26**:477 (Oct.) 1920.

When my associates and I commenced work on the epidemic of poliomyelitis in Baltimore in 1916, we believed definitely that it was a primary disease of the central nervous system, and we were in no way convinced that the paralysis was only a rare complication that might occur in any epidemic. At the end of the epidemic it became clear, however, that our first assumption was not true. Not only from the rarity of the paralysis in this epidemic, but also from the study of the pathology, it became certain that the nerve tissue is not attacked directly, but suffers only from changes in the perivascular spaces, and that in any epidemic these spaces are only rarely involved to any great extent. In the great mass of cases existing in this epidemic, there were only a few patients who ever suffered from any noticeable symptoms of the nervous system. When paralyses did occur, the lesions were not primarily in the nerve tissue, but in the perivascular spaces and the subarachnoid space. The latter fact had already been described in the pathologic studies of Peabody, Draper and Dochez.[7]

PATHOLOGY

Gross Pathology.—The pathologic picture in all of the fatal cases was a general lymphoid hyperplasia which was most marked in the solitary follicles of the gastro-intestinal tract, Peyer's patches and the mesenteric lymph nodes. In each of the fatal cases, the disease began as in the milder ones with fever, gastro-intestinal disturbance, headache and eventual paralysis, which had its origin always in some part of the spinal cord. No death could be attributed to toxic symptoms. Death intervened only when the respiratory center became involved and was eventually the direct result of respiratory paralysis.

In the milder cases only headache, fever and gastro-intestinal disturbances of various degrees were observed.

Portal of Entry in the Gastro-Intestinal Tract.—These general facts led us to believe that the disease begins primarily in the lymphatics of the gastro-intestinal tract and spreads from thence to the lymphatics of other parts. In the less resistant cases it may spread to the subarachnoid space and the perivascular spaces of the spinal cord, medulla and adjacent parts. Wickman[2] thought that it arose in the intestines. Flexner[8] and his students, from the studies of infected monkeys, looked at the nasal passages as the possible portal of entry. In the clinical cases that I observed it was rare to find any very prominent symptoms

7. Peabody, F. W.; Draper, G., and Dochez, A. R.: A Clinical Study of Acute Poliomyelitis, Monogr. Rockefeller Inst. M. Research, New York, 1912.

8. Flexner, S., and Amoss, H. L.: Persistence of the Virus of Poliomyelitis in the Nasopharynx, J. Exper. Med. **29**:379, 1919.

in the nose and throat, and more recently Amoss and Taylor[9] have isolated substances from the secretions of the nasal passages of healthy monkeys that neutralize the virus of this disease.

Microscopic Pathology.—Microscopic examination of the lymph glands shows either a decrease in the blood supply or a dilatation and congestion of the capillary bed, a mild edema which gives the gland a firm resistance and a most active proliferation of the lymphoid cells. The gland becomes a solid mass of lymphocytes or small mononuclear cells. The sinuses are not clearly made out, but the germinal centers of

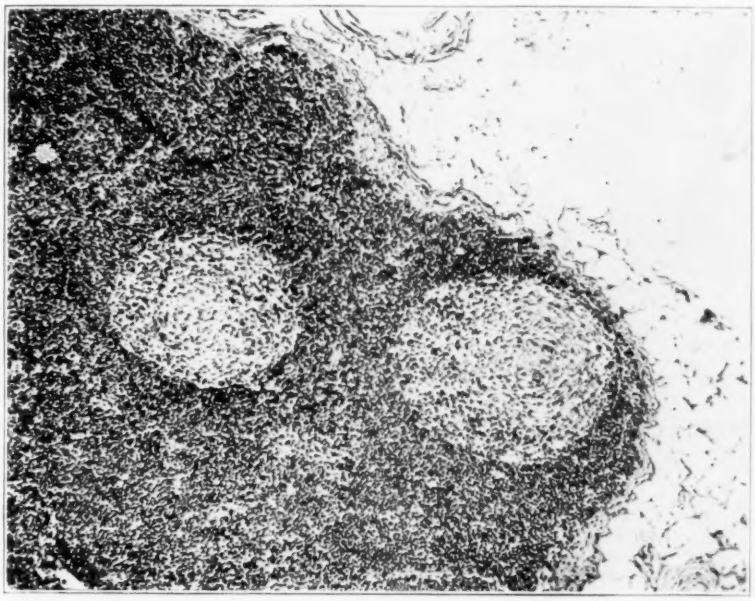

Fig. 1.—A part of a section of a mesenteric lymph gland in a white boy, aged 3½ years. The child was sick for five days, dying of respiratory paralysis. The section shows the large clear centers of the follicles and the proliferation of lymphoid cells peculiar to the condition.

the follicles stand out as prominent, clear areas. These centers show few cells but much cellular débris. There are numbers of mitoses near their peripheries and a few in their more central parts. This growth may proceed to actual necrosis of the centers of these follicles. There is also an active proliferation of the endothelial cells lining the sinuses and other parts of the gland. The picture is that of a true hyperplasia.

9. Amoss, H. L., and Taylor, E.: Neutralization of the Virus of Poliomyelitis by Nasal Washings, J. Exper. Med. **25**:507 (April) 1917.

These changes in the lymphatics become most marked in Peyer's patches, the solitary follicles of the intestines and the mesenteric lymph nodes. These follicles and Peyer's patches are easily seen grossly when the intestines are opened. They are enlarged and raised well above the surface of the surrounding mucous membrane (fig. 2). They show the same picture microscopically as that described for the lymph glands.

While in the fatal cases the enlargement of the lymphatics is most marked in all parts of the body, the mesenteric lymph glands are generally the larger. The malpighian corpuscles of the spleen are prominent on the cut surface of this organ. The peribronchial lymph glands, the lymph glands of the neck, axillae, groin and other parts, and the adenoid tissue of the nose and throat and the thymus show this same hyperplasia

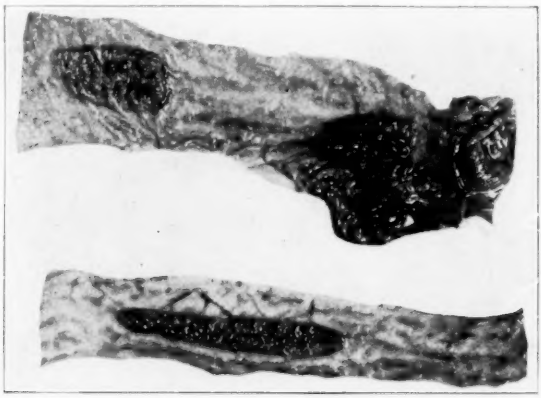

Fig. 2.—The surface of the ileum in a white boy, aged 6½ years. The boy was sick for five days, including an intermission. The figure shows typical hyperplastic Peyer's patches peculiar to this disease.

both grossly and microscopically. While the tonsils were noticeably enlarged in a few cases, it was rare to find them showing the strikingly gross hyperplasia presented by the lymphoid tissue of the gastro-intestinal tract.

Changes in the Nervous System.—The changes in the nervous system were of a similar nature. A primary involvement of the nerve tissue does not exist apparently. The nerve tissue shows changes that are secondary to those existing in the lymphatic channels of this tissue. The primary changes appear to be always in the perivascular spaces and the subarachnoid space. The microscopic picture noted in the subarchnoid space is an active proliferation of the endothelial lining cells following congestion and edema. Many of these cells loosen

and appear as lymphoid cells in this space (fig. 3). Within the nerve tissue, the primary changes are in the perivascular spaces. These spaces become distended with fluid. Their endothelial lining cells proliferate. The edema may become excessive, rupturing the lining membrane, and death may intervene early from this edematous distention alone, if the medulla or the respiratory center is involved (fig. 4).

In other cases, especially in those in which the congestion and edema of the spinal cord persist for a longer time, other changes most often intervene. There is a much greater proliferation of the endothelial

Fig. 3.—A part of a section of the cervical region of the spinal cord and meninges of a white boy, aged 3½ years. The boy was sick for five days and showed only mild meningeal symptoms. The section shows the characteristic changes in the subarachnoid space.

lining cells (fig. 5). The surrounding glia cells may also proliferate and, eventually, areas of necrosis of the nerve tissue result. It is only in the latter areas of necrosis that one ever sees polymorphonuclear cells.

Such abscesses occur always in the immediate vicinity of the blood vessels (fig. 5), and there is considerable variation in the general architecture of the lesion in the different cases. In certain cases there are no marked early changes in the nerve cells, but the proliferation of the

Fig. 4.—A vessel in the anterior horn of the cervical portion of the spinal cord, showing an excessive accumulation of fluid about it and rupture of the perivascular space. The section was taken from a child 2 years and 9 months old who had been sick with the disease for three days, and who died in a convulsion. This child gave a positive Kernig sign.

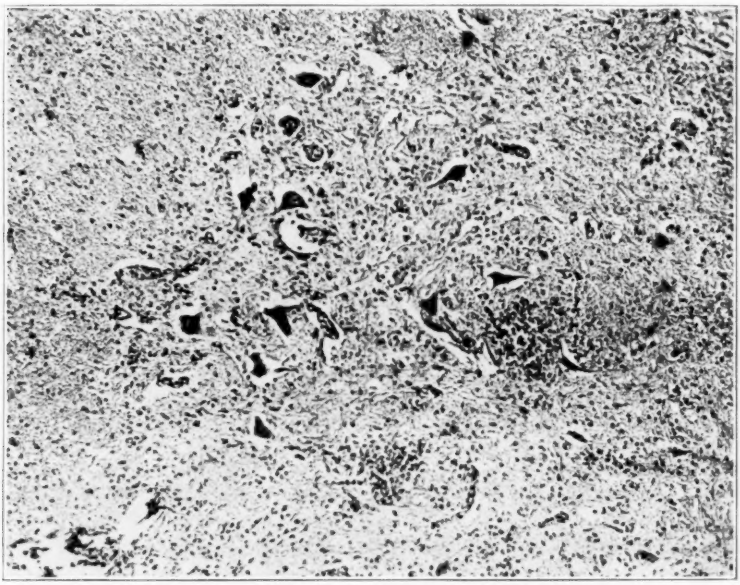

Fig. 5.—A section of the lumbar region of the spinal cord in a colored girl, aged 5 years, who had been sick for four days and had paralysis of the leg for at least forty-eight hours before death. The figure shows secondary changes in the nerve cells and necrosis and the formation of abscesses about a few of the perivascular spaces.

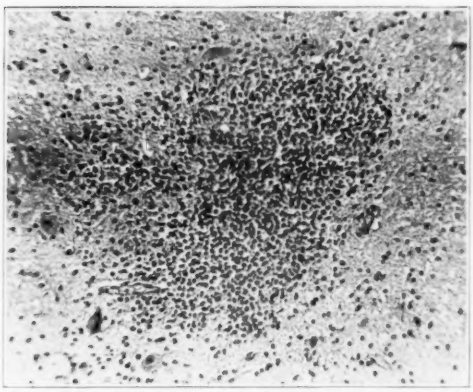

Fig. 6.—Blood vessels of the lumbar region of the spinal cord in a white boy, aged 3 years. The child had been known to be sick for only three days. The section shows an excessive proliferation of endothelial cells about several of the smaller blood vessels.

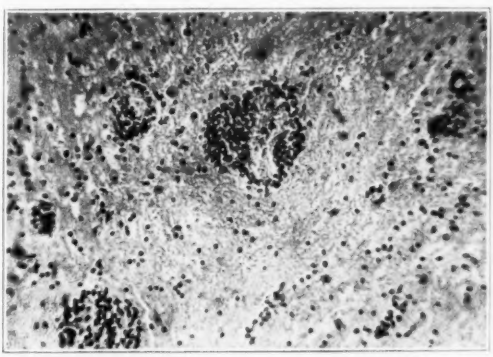

Fig. 7.—Vessels in a section of the cervical region of the spinal cord in a colored girl, aged 4 years, who had been sick for five days. The upper extremities of this child had been paralyzed forty-eight hours before death. The section shows an active proliferation of the endothelial cells of the perivascular spaces without edema and some proliferation of the glia cells.

endothelial lining cells of the perivascular spaces becomes excessive. These cells not only distend the space, but they spread to fill the adjacent nerve tissue (fig. 6). In other cases the spaces merely become distended with these cells, the cell breaking the wall of the space at only a few places (fig. 7). The glia cells may or may not show evidence of proliferation in either of these types.

In a third group, the picture may be different. The proliferating endothelial cells migrate directly into the nerve tissue. The space is often devoid of fluid. The nerve tissue about the vessel shows fatty changes, and there is an evident proliferation of the adjacent glia cells (fig. 8).

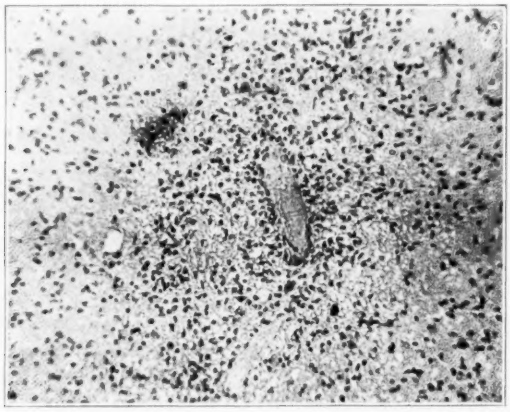

Fig. 8.—A section taken from the cervical region of the spinal cord of a white boy, aged 7½ years, who had been sick for five days, including a remission. The upper extremities of this child were paralyzed two and a half days before death. The proliferating endothelial cells have migrated into the nerve tissue. The nerve tissue shows fatty changes, and there is very evident proliferation of the glia cells.

While Hurst [10] has tried to show that the primary lesions of the central nervous system are in the nerve cells rather than in the lymphatic channels, it seems evident that he drew these conclusions because he was looking at the end-result of a chain of pathologic processes rather than at the primary lesion. He did not study the disease in man but in monkeys, and in monkeys that had been inoculated with the virus and in many of which this virus had been introduced directly into the central nervous system. Monkeys are very susceptible to the virus, and the destruction is often much more marked in their nerve tissue than in

10. Hurst, E. W.: The Histology of Experimental Poliomyelitis, J. Path. & Bact. **32**:457, 1929.

that of human beings. Any evidence of a primary destruction of the nerve cells was absent in our case, and a careful survey of Hurst's description disproves his deductions: the lesions that he described in the brain at the border of the chief parts involved showed only involvement of the perivascular spaces and no change in the nerve cells.

Clark and Amoss [11] had already shown that when monkeys are infected by an intraspinal injection, the meninges may become infected without paralysis, showing, as they stated, that the changes in the interstitial tissue in the meninges, blood vessels and ground substance play a determining part.

A Disease of the Lymphatic Tissues.—It seems quite evident, therefore, from all available evidence at the present time that the primary lesions of the poliomyelitis are not in the central nervous system. Poliomyelitis is probably a misnomer as it is applied to the disease as a whole. It is merely a complication of a widespread infectious disease. This disease is not a primary disease of the central nervous system; it is a disease of the lymphatic systems of the body. The lesions presented are not of the kind in which pus appears early, but the lesion is rather that of an acute hyperplastic lymphadenitis.

While it is possible that the disease may be made to enter monkeys by way of the nasal passages, in the greater number of cases in man the disease enters apparently by way of the intestinal or gastro-intestinal tract. Clinically, in the great majority of cases, the disease begins as a gastro-intestinal upset, and pathologically, the most pronounced lesions are usually found in the solitary follicles and Peyer's patches of these parts. From these lymphatic structures it spreads to other parts of the body until the immunity of the patient overcomes its activity. At the present time, the great majority of persons recover after a small involvement of these tissues, while in others the lymphatics throughout the entire organism become involved, and paralysis or death may intervene.

DIFFICULTIES ENCOUNTERED IN COMBATING THE DISEASE

Inadequacy of Present Methods of Quarantine.—I bring these facts forward because it seems that the present general attitude and methods of quarantine can have little influence in stopping the spread of this infection. Poliomyelitis, as it is now termed, is probably always present as a sporadic disease, and it appears in epidemic form as often as it is carried into isolated districts or a new crop of children develops in the older communities. This deduction has been borne out not only by clinical studies, but by recent experimental evidence. Paralyses are more common in the northern than in the southern parts of this country.

11. Clark, P. F., and Amoss, H. L.: Intraspinous Infection in Poliomyelitis, J. Exper. Med. **19**:217, 1914.

Epidemics are more common in the cities than in the country. Aycock and Kramer [12] studied the blood of adults in both urban and rural populations, who, so far as they knew, had never had the disease. The blood of these persons was tested on monkeys for their ability to neutralize a virulent virus. The blood of as many as 69 per cent of those from the cities neutralized the virus, while only 20.7 per cent of those from country districts showed the existence of such immune bodies. In the same manner, these authors [13] studied the blood of adults from cities in the South. They found as many immune there as in the North, showing

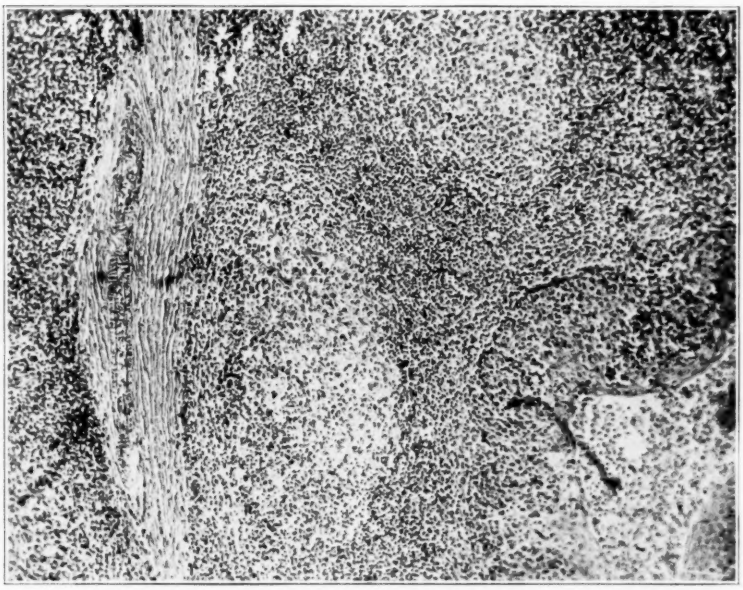

Fig. 9.—A section of the tonsil of a white boy, aged 6½ years, who had been sick for four days. It shows the typical hyperplasia seen in other lymphoid tissues of the body.

that the disease must exist there as elsewhere, but that fewer show complications of the central nervous system.

It thus becomes evident that if this disease is to be eradicated from the community, it must be attacked in a manner different from that used

12. Aycock, W. Lloyd, and Kramer, S. D.: Immunity to Poliomyelitis in Normal Individuals in Urban and Rural Communities as Indicated by the Neutralization Test, J. Prev. Med. **4**:189 (May) 1930.

13. Aycock, W. Lloyd, and Kramer, S. D.: Immunity to Poliomyelitis in a Southern Population as Shown by the Neutralization Test, J. Prev. Med. **4**:201 (May) 1930.

at the present time. It is evidently a widespread infection. To protect any person against it would necessitate complete isolation throughout his entire life. As Frost [14] pointed out, the cases in which paralysis occurs are generally widely separated, and it is of little use to quarantine these patients while there are probably hundreds of others at large carrying germs as virulent as do those who have suffered permanent disability.

Infantile Paralysis a Misnomer.—The name infantile paralysis is also misleading. While it is true that children are most often attacked, 90 per cent of the cases appearing probably in children from 1 to 10 years of age (Parkes and Neal [1]), adults are not immune unless they have already come in contact with the virus. The high incidence in children is probably due only to the fact that the disease is most contagious and very widespread. It is similar to measles in this regard. Most adults escape in any epidemic because they have suffered from the disease at a previous time. One attack seems to be ample to produce immunity. Frost [14] noted in an epidemic in New York City that sixty-five children to one adult were affected. In the epidemic in Iowa, on the other hand, where the population is more scattered and the chances of previous infection are less, there were only six children sick to one adult.

Difficulty of Diagnosing Mild Cases.—The difficulty of following accurately the course of any epidemic of poliomyelitis is the mild character of most cases and the difficulty of distinguishing them from any other form of gastro-intestinal disturbance. In a careful study of an epidemic in an apartment house in Baltimore, which Park and I were able to make in 1916,[15] we found that every child became ill who had previously come in contact with the first child infected or with others infected from him. While there seemed to be little doubt that each of these children had the disease, it would have been difficult to prove its existence in many of them if others had not shown involvement of the central nervous tissue.

This difficulty of making a diagnosis in the mild forms of the disease, yet the evident widespread character of the infection, is well illustrated in the recent epidemic in California. A large number of cases of gastro-intestinal infection existed in the small vicinity about my own residence. Two of my own children became ill with headache, vomiting and fever, the indisposition lasting one day in one case and two days in the other. The wife of a neighbor, a woman 31, had been ill nine days previously with vomiting and headache. She recovered in a few days. Three days

14. Frost, W. H.: The Epidemiology of Poliomyelitis, J. Iowa M. Soc. **19**: 223 (May) 1929.

15. Burrows, M. T., and Park, E. A.: The Study of a Small Outbreak of Poliomyelitis in an Apartment House, Occurring in the Course of an Epidemic in a Large City, Arch. Int. Med. **20**:56 (July) 1917.

after the onset of her trouble, her husband became ill with something peculiar to his experience. The onset of his illness was sudden. His temperature rose to 102.5 F., and he suffered from a severe and peculiar headache. They had been patients of mine, and I was called to see him. The history revealed an obstinate constipation, also. The examination showed very little or no coating of the tongue, but slight enlargement of the cervical, inguinal, axillary and epitrochlear glands. The abdomen was slightly distended, but one could with slight pressure feel small gas pockets over the whole of it. A diagnosis of poliomyelitis seemed most logical. A saline cathartic was ordered.[16] The temperature went down promptly. The patient was quite ill for five days, and he remained weak and exhausted for another twelve days.

COURSE OF THE DISEASE

Period of Incubation.—In this small epidemic about my own residence in California, the period of incubation was, I calculated, from three to five days. Aycock and Luther [17] gave the period of incubation as from six to twenty days. In the apartment house in Baltimore, where all contacts could be followed carefully, Park and I [15] concluded that the period of incubation was from three to seven days.

Paralysis, a Complication.—While it is true that infectious diseases of all types exist in a mild form in many persons and that others apparently escape them entirely, the picture in poliomyelitis is somewhat different. The disease always begins as a systemic disturbance. The great majority of the cases are mild. Paralysis is evidently a rare occurrence. The paralysis thus appears to be a complication rather than a primary part of the condition. It is similar to the nephritis that may complicate scarlet fever. While the symptoms of the central nervous system may follow the systemic symptoms at once in a few cases, there is generally a remission between the systemic symptoms and the paralysis.[18]

Transmission.—The disease is probably transmitted from patient to patient by direct contact. The germs may be carried in the mouths of

16. Edema is one of the striking changes in the tissues in this disease. Salts are indicated because the treatment should be directed toward relieving this edema and the resulting pressure. Aycock and Amoss have noted some results after the injection of hypertonic solution of salt together with serum in an attempt to cure the disease in monkeys. (Aycock, W. Lloyd, and Amoss, H. L.: Experiments on Local Specific Therapy in Poliomyelitis: Utilization of Hypertonic Solutions in the Serum Treatment of Experimental Poliomyelitis, Bull. Johns Hopkins Hosp. **34:** 361 [Nov.] 1923.)

17. Aycock, W. Lloyd, and Luther, Eliot H.: The Incubation Period of Poliomyelitis, J. Prev. Med. **3:** 103 (March) 1929.

18. Peabody, Draper and Dochez (footnote 7). Leake, J. P.: The Diagnosis of Poliomyelitis, Pub. Health Rep. **32:** 1831 (Nov. 2) 1917.

healthy persons [19] as well as in the mouths of patients. Patients harbor the germs for at least several days after having apparently recovered completely. Park and I found what appeared to be evidence of the germs in the mouth of one patient nineteen days after complete recovery. There was no evidence of the disease in the nasal and pharyngeal washing of this patient and of another patient thirty-five days after they had recovered from the disease (Burrows and Park [15]).

Prevalence.—As I have already stated, poliomyelitis is evidently as widespread as any infectious disease. It is evidently present at all times in thickly populated districts and occurs in epidemic form as soon as a new crop of children of considerable number develops in that district. In many of the larger eastern cities these epidemics occur more or less regularly every two to six years, and the high incidence is in children from a few months to 6 years of age. The question of escaping paralysis is evidently a question of one's resistance to the disease.

In the epidemic of 1916, as many cases in which paralysis occurred existed as in any epidemic. Even in this epidemic we calculated that within the field of our observation there were 1,500 cases in which paralysis occurred, 250 in which it did not occur and 50 cases terminating in death. In the cases that we studied we found a group of children well isolated for a time from the infection. The disease was introduced to one family finally, however, by an adult carrier. Every child in the apartment house who came in contact with this family or with children who had made this contact became ill, while those who did not make these contacts remained well.

In this particular isolated group, only two of the twelve children who were ill became paralyzed. Muscular weakness was present for a time in a few; others showed meningeal symptoms, and others only headache, fever and gastro-intestinal disturbance of short duration.

CLINICOPATHOLOGIC CORRELATIONS

In the cases that came to autopsy the pathologic changes were as I have outlined them. There was a general lymphoid hyperplasia reaching its maximum in Peyer's patches, the solitary lymph follicle of the gastro-intestinal tract and the mesenteric lymph nodes. In many of the cases, no lymphoid tissues escaped except perhaps the perivascular spaces of the brain itself; in others, the spread was more irregular and patchy. Only a part of the lymphoid tissue was involved, and only parts of the spinal cord, pons and medulla showed changes.

19. Flexner, S.; Clark, P. F., and Fraser, F. R.: Epidemic Poliomyelitis: Fourteenth Note: Passive Human Carriage of the Virus of Poliomyelitis, J. A. M. A. **60**:201 (Jan. 18) 1913.

Careful clinical studies have divided the cases into several groups. One classification that may be given is as follows:

Mild	Meningeal	Ataxic
Abortive	Paralytic	Cerebral
	Landry's	

No cerebral type of case was encountered by us in the Baltimore epidemic or elsewhere, although such types have been described in other epidemics. Ataxic types, on the other hand, were seen. In the fatal cases that came to autopsy we found no involvement of the cerebral or cerebellar cortices. The basal nuclei of the brain also showed no evidence of the disease, while the dentate nucleus of the cerebellum was involved in about one third of the cases. We were inclined to believe, therefore, that the ataxia in certain cases might indicate an involvement of this nucleus without injury to the respiratory center.

The ganglions of the dorsal roots of the spinal and the cerebral nerves frequently showed changes, while the nerves themselves were always free from inflammatory changes. In the three cases of epidemic encephalitis that I studied,[6] the picture was different. There was involvement of the cerebral cortex and the basal nuclei of the brain. The cranial nerves also always showed inflammatory changes. These peculiarities separated the cases of epidemic encephalitis from poliomyelitis.

As Leake [18] clearly pointed out, the systemic changes that are present in all cases are fever, headache and gastro-intestinal disturbances. The latter disturbances, which have been noted most often, are constipation and slight distention. Diarrhea has been seen in certain epidemics, however. Sore throat may be present but it is not common, as are the gastro-intestinal symptoms. The common picture seen at the onset of the disease in this country has been headache, fever (generally pronounced), constipation and vomiting.

We found the tonsils involved in all fatal cases, but this involvement was not as pronounced as that seen in the Peyer's patches and the solitary follicles of the intestines. The tonsils showed microscopically the same hyperplasia, however, as that seen in other lymphatic tissue (fig. 9).

In more than half of the fatal cases in which I performed an autopsy the whole of the spinal cord showed pathologic changes. These changes were most advanced in the lower part of the cord. The picture in these cases was that of Landry's paralysis. In the other cases, the lesions were focal, one part of the cord being involved in one case and another in others. The muscles most frequently involved in the nonfatal cases were the gastrocnemius in the legs and the deltoid in the arms. Death intervened in two of the fatal cases apparently from an immediate involvement of the upper cervical cord and the medulla.

As Leake [18] also pointed out, the meningeal symptoms when present are not so pronounced as those seen in tuberculous and other infections

of the meninges. One might draw this conclusion from a comparison of the pathologic pictures alone. The patients in the epidemic in Baltimore who showed marked meningeal symptoms and who succumbed later and were brought to autopsy were found to have been suffering from tuberculous meningitis.

SUMMARY

While the exact portal of entry of poliomyelitis is not known, from the clinical and pathologic studies available it seems that the disease must enter by way of the gastro-intestinal lymphatics (Peyer's patches and the solitary follicles). Symptoms in the latter region are always present, while symptoms in the throat are not common. From this portal of entry the disease spreads to contiguous lymphatic tissues in the body. In the mild cases, immunity and recovery result after a limited amount of this tissue is involved. In the less resistant cases, wider extension results, even to the involvement of the lymphoid structures of the central nervous system.

In this regard the disease resembles other infectious diseases. When a number of persons are infected, for instance, with streptococcal sore throat, in one the disease ends after an involvement of the throat alone; in another it spreads from the throat to the larynx and trachea; in another to the bronchi, and in others pneumonia and death result. So in infantile paralysis, it appears that, in the great mass of cases, immunity develops with involvement of small parts of the peripheral lymphatic tissue; in others, more of this tissue is involved, so that one may palpate the cervical, axillary, inguinal and even the epitrochlear and popliteal lymph glands; in still others, the infection spreads to the lymphatic structures of the nerve tissue.

While there was a time when it was thought that poliomyelitis was peculiar in that cases of it are often widely separated, it has now become known that this is not true. It is evidently only a complication in a widespread infectious disease. Most persons show a striking resistance to this infection. An involvement of the gastro-intestinal lymphatics alone is the picture seen in most of them. Many others do not show these symptoms to an extent sufficient to stop their ordinary pursuits of life.

It is in the light of these facts and the pathology as it is known that I have suggested that the name infantile paralysis be changed. It is possible that such a change in the name and the new attitude that it may create will lead to a more rapid advance in the knowledge of the nature of the disease. Such a change in name may also create a more understanding attitude toward it on the part of the laity and correct some of their false impressions. At the present time the methods of quarantine are evidently inadequate and probably quite useless in most instances.

Since it appears that this disease is as widespread as measles, and since the great majority of cases are mild, it does not seem likely that a sufficient number of cases can be recognized to stop any epidemic by quarantine. It is evident, therefore, that other methods must be devised. It seems necessary from the clinical and pathologic studies already made that some method be devised by which the resistance of the whole population can be raised. Some method of vaccination or inoculation such as that used in diphtheria may be the means by which the hideous aspects of this disease can be removed. A mild infection with poliomyelitis seems to leave no bad effects. It is the paralysis or paralysis and death that await the few in every community that must be guarded against.

CONCLUSIONS

1. The pathology of poliomyelitis has been described as studied in a series of about fifty autopsies.

2. The present-day attitude of students of the clinical aspects of this disease has been reviewed with certain original observations.

3. The name acute lymphatic hyperplasia has been suggested for this disease in order that all cases may be properly included under the name. The names "infantile paralysis" and "poliomyelitis" designate only a few of the cases existing at any time in the community.

4. Evidence is brought forward to show that the present methods of quarantine are inadequate. Some method of inoculation or some other measure must be devised to raise the resistance of the whole population if the few are to escape paralysis and death from it.

94 North Madison Avenue.

EXPERIMENTAL EDEMA *

SAMUEL A. SHELBURNE, M.D.
DALLAS, TEXAS
AND
WILLIAM C. EGLOFF, M.D.
BOSTON

The Starling hypothesis, which holds that the exchange of water between the blood and the tissue fluids through the capillary wall is determined by a delicate balance between the hydrostatic pressure in the capillaries and the osmotic pressure of the plasma proteins, has been almost definitely established by research work, both experimental and clinical. In 1926, Landis [1] measured the capillary pressure by introducing the micropipet into the capillaries of the mesentery of the frog, and found a gradient of pressure ranging from 14.5 cm. of water at the arterial end to 10 cm. at the venous end. He found that filtration outward occurred when the pressure was above 11.5 cm. of water. White [2] found the osmotic pressure of the plasma proteins of the frog to be from 10 to 12 cm. of water. Recently Landis [3] (1930) measured the capillary blood pressure in human skin and found that it ranged from 32 mm. of mercury in the arteriolar limb to 12 mm. of mercury in the venous limb. The osmotic pressure of plasma proteins in man ranges from 23 to 28 mm. of mercury. Leiter [4] (1928) added further evidence when he produced edema by lowering the plasma proteins of dogs. This was accomplished by the selective removal of the plasma (plasmapheresis). Similar observations were published by Barker and Kirk [5] (1930).

The clinical evidence has been of no less value. Csatary [6] (1891) noted that there was a decrease in the plasma proteins in some patients with nephritis and that the albumin was lowered more than the globulin. These observations were confirmed by Linder, Lundsgaard and Van

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* From the Medical Clinic of the Peter Bent Brigham Hospital and the Medical Laboratories of the Harvard Medical School.

1. Landis, E. M.: The Capillary Pressure in Frog Mesentery as Determined by Micro-Injection Methods, Am. J. Physiol. **75**:548, 1926.

2. White, H. L.: On Glomerular Filtration, Am. J. Physiol. **68**:523, 1924.

3. Landis, E. M.: Micro-Injection Studies of Capillary Blood Pressure in Human Skin, Heart **15**:209, 1930.

4. Leiter, Louis: Experimental Edema, Proc. Soc. Exper. Biol. & Med. **26**: 173, 1928.

5. Barker, M. H., and Kirk, E. J.: Experimental Edema, Arch. Int. Med. **45**:319 (March) 1930.

6. Csatary, A.: Ueber Globinurie, Deutsches Arch. f. klin. Med. **48**:358, 1891.

Slyke [7] (1924), as well as by others. With Starling's hypothesis in mind, Epstein [8] expressed the belief that the edema in nephrosis is due to the low plasma protein, and he introduced the diet high in protein to correct this deficiency. This work was confirmed by Moore and Van Slyke [9] (1903).

We started the experiments reported here with the purpose of producing edema by plasmapheresis in order to study the effect of electrolytes on edema. We adopted essentially the method used by Barker and Kirk [5] in this laboratory last year. This method of bringing about edema depends on the daily removal of a large amount of blood plasma. Reaccumulation of the plasma protein is prevented by giving a diet deficient in protein. As a result of this reduction in plasma proteins, the osmotic pressure which these proteins exert falls below the capillary pressure, and fluid passes into the tissues. Experiments on three dogs are reported here. The dogs were fed a diet low in protein but adequate in carbohydrate and fat (diet A).[10] The first dog (dog 1) was used purely as a control on the diet, but after a long period on this diet, the plasma proteins fell so low that the addition of salt and water administered by stomach tube precipitated a massive edema. Edema was produced by diet and plasmapheresis in the other two dogs (dogs 2 and 3). The action of various salts on both types of edema was studied. A comparison is drawn between the pathologic and chemical observations in these two types of edema.

PRODUCTION OF EDEMA IN A DOG BY A DIET LOW IN PROTEIN BUT ADEQUATE IN CARBOHYDRATE AND FAT

Schittenhelm and Schlecht [11] studied the edema of malnutrition and found the serum proteins low. Denton and Kohman [12] (1913) found

7. Linder, G. C.; Lundsgaard, C., and Van Slyke, D. D.: The Concentration of the Plasma Proteins in Nephritis, J. Exper. Med. **39**:887, 1924.

8. Epstein, A. A.: Concerning the Causation of Edema in Chronic Parenchymatous Nephritis: Method for Its Alleviation, Am. J. M. Sc. **154**:638, 1917.

9. Moore, N. S., and Van Slyke, D. D.: The Relationships Between the Plasma Specific Gravity, Plasma Protein Content and Edema in Nephritis, J. Clin. Investigation **8**:337, 1930.

10. Diet A:

	Grams	Calories	Protein	Total Base	Chlorides
Baked potato	50	42	1.10	0.246	0.019
Cream	50	193	1.25	0.128	0.040
Turnip	200	41	2.60	0.950	0.041
Butter (unsalted)	25	199	0.25	0.028	0.030
Lactose	60	240	....	....	....
		715	5.20	1.352	0.130

11. Schittenhelm, A., and Schlecht, H.: Ueber Oedemkrankheit mit hypotonischer Bradykardie, Berl. med. Wchnschr. **55**:1138, 1918.

12. Denton, M. C., and Kohman, E. A.: Feeding Experiments with Raw and Boiled Carrots, J. Biol. Chem. **36**:249, 1918.

that edema developed in rats fed on carrots. Frisch, Mendel and Peters[13] (1930) confirmed these experiments and revealed that the total plasma proteins were low in their rats. They also made clear that vitamin deficiency played no part in the edema produced in this way. We have been unable to find any reference to the production of edema in dogs by the use of diets deficient in protein.

Dog 1 was chosen at the beginning of our experiments as a control animal for a diet which would be most favorable for the production of edema by plasmapheresis. However, after she had been fed this diet for eighty-eight days, the plasma proteins fell so low that we were able to precipitate massive edema by the addition of large amounts of water and sodium chloride by stomach tube. She was given an equal amount of water and salt at one period before the level of the plasma protein fell. No edema appeared, and there was no increase in the weight. The observations made on this dog are given in detail in the following protocol.

Dog 1.—This dog was a healthy, well nourished female bull terrier, weighing 16.9 Kg. She was given diet A every day for a period of ninety-eight days, and at no time was plasmapheresis performed. The determinations of the total plasma protein, fibrinogen, albumin and globulin were done by the combined methods of Howe,[14] Wu[15] and Koch and McMeekin,[16] as described by Hawk and Bergheim,[17] that for the blood cholesterol as described by Bloor, Pelkan and Allen,[18] that for the blood chloride by the blood method of Whitehorn[19] and that for the blood creatinine by the method of Folin;[20] determinations were also made with the hematocrit. All determinations were made on arterial blood collected in tubes containing 3 mg. of sodium oxalate per cubic centimeter of blood drawn. The dog was placed in a metabolism cage, and the urine collected over periods of two and three

13. Frisch, R. A.; Mendel, L. B., and Peters, J. B.: The Production of Edema and Serum Protein Deficiency in White Rats by Low Protein Diets, J. Biol. Chem. **84**:167, 1929.

14. Howe, P. E.: The Determination of Proteins in the Blood; A Micro Method, J. Biol. Chem. **49**:109, 1921.

15. Wu, Hsien: A New Colorimetric Method for the Determination of Plasma Proteins, J. Biol. Chem. **51**:33, 1922.

16. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler Folin Reagent for Ammonia, J. Am. Chem. Soc. **46**:2066, 1924.

17. Hawk, P. B., and Bergheim, O.: Practical Physiological Chemistry, ed. 9, Philadelphia, P. Blakiston's Son & Company, 1926.

18. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma, J. Biol. Chem. **52**: 191, 1922.

19. Whitehorn, J. C.: Simplified Method for the Determination of Chlorides in Blood, J. Biol. Chem. **45**:449, 1921.

20. Folin, O.: Laboratory Manual of Biologic Chemistry, ed. 4, New York, D. Appleton & Company, 1927, pp. 165 and 243.

days was analyzed for chloride and creatinine by the methods of Folin [21] and for total nitrogen by the method of Folin and Denis.[22] Fresh specimens of urine were collected frequently for routine clinical analysis. The preliminary control studies gave normal results (table 1).

The dog consumed an average of about 75 per cent of the diet. She lost weight gradually, showing progressive weakness and muscular wasting, but she otherwise remained in good health throughout the experiments, except for a corneal ulcer which developed during the last two weeks. The rectal temperature varied from 101 to 102 F., which is within the normal range for dogs. After she had been on diet A for twenty-eight days, the total plasma protein had dropped from 6.8 to 4.7 Gm. per hundred cubic centimeters, and the albumin-globulin ratio had changed from 4:2.2 to 1.8:1.6. The fibrinogen changed from 0.6 to 0.3 Gm. per hundred cubic centimeters. At this time, the dog was fed 13.5 Gm. of sodium chloride in 1,600 cc. of water by stomach tube daily for three days. The solution was isotonic and did not cause diarrhea. The total output of urine for the three days was 4,500 cc., and practically all the chloride was excreted. The output of creatinine was also normal. There was no edema present and no gain in weight.

On the eighty-third day, the total plasma protein had fallen to 3.2 Gm. per hundred cubic centimeters, and the albumin-globulin ratio was reversed, i. e., 1.3:1.7. At this time the same amounts of sodium chloride and water used before (13.5 Gm. in 1,600 cc. of water) were given daily for three days. Definite pitting edema was noticed at the end of the first day, and at the end of the third day subcutaneous edema was marked. Ascites was easily demonstrable. The dog's weight had increased 1.9 Kg. during the three days. About half the chloride was excreted.

Immediately following this period, beginning on the eighty-sixth day, the dog was given 800 cc. of water with no added salt for two days. On this regimen she lost weight and the edema decreased, but did not disappear. On the eighty-eighth day, the edema and weight were again increased by the addition of sodium chloride and water. The next day, 1,600 cc. of water alone was added, and the animal lost weight. Then for the next two days she was given 11 Gm. of potassium chloride in 1,200 cc. of water by stomach tube. There was no increase in edema or weight; in fact, she lost 0.8 Kg. Following this period, sodium chloride and water were again administered, and the usual increase in edema and weight occurred.

The cholesterol remained normal except for two determinations on the seventy-seventh and eighty-fourth days, when the readings were 417 and 409, respectively. Both of these readings were controlled by determinations on normal blood. The blood pressure did not vary from the normal. Frequent routine urinalyses showed no abnormalities.

The dog was killed on the ninety-eighth day by the intravenous injection of 10 cc. of ether, and autopsy was performed. There was moderate subcutaneous edema, and 600 cc. of slightly opalescent fluid was removed from the peritoneal cavity. The specific gravity of this fluid was 1.005, the total protein, 0.17 per cent, and there was 410 mg. of chloride per hundred cubic centimeters. The muscles were normal in color and consistency, but were greatly wasted. The left

21. Folin, O.: On the Determination of Creatinine and Creatine in Urine, J Biol. Chem. **17**:469, 1914.

22. Folin, O., and Denis, W.: Nitrogen Determination by Direct Nesslerization, J. Biol. Chem. **26**:486, 1916.

TABLE 1.—*Results of Studies on Dog 1**

Date	Wt.	Hem.	T.P.	Alb.	Glob.	Fib.	Chol.	B.U.N.	Bl. Cr.	Bl. Cl.	Duration of Metabolism Experiment, Hours	Total Intake of Water, Cc.	Total Output of Urine, Cc.	Ur. Cr.	Ur. Cl.	Ur N.	Ur. Alb.	Edema	Total Intake of Salt	Diet	Comment
2/28/30	16.9	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	A	Excellent health
3/ 3/30	16.9	48.5	6.8	4.0	2.2	0.6	166	8	...	290	72	ad lib.	850	544	0.15	3.2	0	0	.....	A	
3/14/30	16.6	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	..	0	.....	A	
3/18/30	16.4	46.3	5.1	2.7	1.8	0.6	218	8	...	...	96	ad lib.	650	325	0.24	...	0	0	.....	A	
3/24/30	16.0	48.0	5.4	2.6	2.5	0.3	276	..	0.6	280	..	ad lib.	.....	...	....	...	..	0	.....	A	
3/27/30	15.5	47.0	4.7	1.8	2.6	0.3	276	..	...	300	72	4,800	4,500	340	7.83	2.6	0	0	NaCl 39 g.	A	No signs of edema
5/ 8/30	13.5	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	A	
5/11/30	13.3	....	...	...	...	...	...	..	0.6	295	72	ad lib.	820	195	0.24	...	0	0	.....	A	
5/22/30	13.1	42.0	3.2	1.3	1.7	0.2	409	6	0.6	325	..	ad lib.	.....	...	....	...	..	0	.....	A	
5/25/30	15.0	33.5	3.6	1.5	1.7	0.4	260	4	...	340	72	4,800	2,400	...	4.24	3.1	0	+++	NaCl 39 g.	A	Edema marked
5/27/30	13.9	....	...	...	...	...	...	..	...	...	48	1,600	2,400	198	2.88	2.4	0	+	.....	A	Moderately weak
5/28/30	13.3	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	..	+	.....	A	
5/30/30	13.8	....	...	...	...	...	...	..	...	...	48	2,400	1,700	187	5.6	2.9	0	+++	NaCl 22 g.	A	Edema marked
5/31/30	12.9	30.5	2.9	1.2	1.4	0.2	229	..	...	300	..	ad lib.	.....	...	....	...	..	++	.....	A	
6/ 2/30	12.1	....	...	...	...	...	...	..	...	...	48	2,400	2,100	175	5.7	0.9	0	0	KCl 22 g.	A	No edema
6/ 3/30	12.1	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	A	Weakness, marked muscular wasting
6/ 5/30	13.9	30.0	4.27	1.3	2.4	0.5	240	5	0.6	340	48	2,400	675	185	1.77	2.1	0	+++	NaCl 22 g.	A	Killed (10 cc. ether intravenously)

* An interpretation of abbreviations in this and the following tables follows: Wt., weight in kilograms; Hem., hematocrit, percentage of red blood cells; T.P., grams of plasma protein per hundred cubic centimeters of blood; Alb., grams of plasma albumin per hundred cubic centimeters of blood; Glob., grams of plasma globulin per hundred cubic centimeters of blood; Fib., grams of fibrinogen per hundred cubic centimeters of blood; Chol., milligrams of cholesterol per hundred cubic centimeters of blood; B.U.N., milligrams of urea nitrogen per hundred cubic centimeters of blood; Bl. Cr., milligrams of creatinine per hundred cubic centimeters of blood; Bl. Cl., milligrams of chloride per hundred cubic centimeters of blood; Ur. Cr., milligrams of creatinine in urine per twenty-four hours; Ur. Cl., grams of chloride in urine per twenty-four hours; Ur. N., grams of nitrogen in urine per twenty-four hours, and Ur. Alb., albumin in urine.

kidney weighed 42 Gm., the right 43 Gm. Grossly, the kidneys appeared normal. The capsules stripped with slight difficulty, leaving a smooth surface. The ratio of the width of the cortex to that of the medulla was normal. The bladder urine showed no albumin, and the sediment showed only an occasional white blood cell. The other organs were normal in weight and appearance.

The microscopic sections were examined by Dr. R. Z. Schulz and Dr. S. B. Wolbach. Microscopic sections of the kidneys, stained with eosin-methylene blue (methylthionine chloride, U.S.P.) and scarlet red, showed a fatty degeneration of the collecting and the distal convoluted tubules and a slight degree in the proximal tubules. This involvement was of moderate degree. The nuclei of the involved tubules were not pyknotic. Occasional loops of Henle contained a few cells with fine fat droplets. A few hyaline casts were present. The glomeruli were of normal appearance, with no evidence of inflammatory cell infiltration, proliferation of the epithelium, fibrosis or deposit of fat either in the intracapsular space or about the capsules. The stroma of the kidney was not increased. Aside from the fatty degeneration, there were no areas suggesting either a healed or an active lesion. The degree of fatty change was more marked than in dog 2.

The liver showed a moderate degree of fatty infiltration which was in both the central and the portal areas; the latter, however, were more extensively involved. The majority of the cells had hypochromatic cytoplasm which was due to fat vacuoles, but in others it was due to the poor staining qualities of the cytoplasm. A considerable number of Kupffer cells filled with a brownish pigment were found in the sinuses. The portal spaces were quite normal in appearance, and there was no fibrosis or infiltration with inflammatory cells.

COMMENT

This animal was the only one used as a control for the diet, so at present we are able to report on only one experiment of this type. Further work will be necessary to establish the fact that such a diet will produce edema in most dogs. Since so much depends on the intake of protein, it may be worth while to emphasize again the fact that this dog ate only three fourths of the diet each day. We believe that this method of producing edema in dogs offers a splendid means for the study of the various problems of this subject.

The microscopic sections of the kidney showed fatty degeneration of the collecting and distal convoluted tubules. These observations are identical with those in the sections from the kidneys of the dogs in which edema was produced by plasmapheresis (see protocols of dogs 2 and 3). There was no evidence of glomerular nephritis in these tissues. The liver also showed fatty infiltration.

There was no evidence of renal insufficiency, the urea nitrogen of the blood remained normal, and there were no urinary abnormalities. The rise in the cholesterol at one period is interesting. This has been observed in dogs that have been starved over a long period of time.[23] We checked the diet for vitamin deficiency, and it was apparently ade-

23. Muller, Gulli Lindh: The Cholesterol Metabolism in Health and Disease, Medicine **9**:119, 1930.

quate except for vitamin E (antisterility). The corneal ulcer seemed to be traumatic. It certainly could not be related to a deficiency in vitamin A, as this vitamin is most abundant in butter and cream.

The results of the experiments on the effects of sodium chloride and potassium chloride on the edema of dog 1 are discussed in the next section.

THE INFLUENCE OF CERTAIN ELECTROLYTES ON EXPERIMENTAL EDEMA

The importance of the electrolytes in the edema of patients with cardiac and with renal disease was realized by Widal [24] (1903), when he advocated a diet with a low content of sodium chloride for the correction of this condition. Widal considered the chloride ion as responsible for the retention of the water. Blum [25] (1909) showed that it is probably the sodium ion that the kidney has difficulty in excreting. Blum and his associates [26] (1921), Magnus-Levy [27] (1920) and Pfeiffer [28] (1911) found that edema in patients is increased by the ingestion of sodium chloride, sodium bicarbonate or sodium bromide, but that potassium chloride, ammonium chloride or calcium chloride fails to increase the edema. These results strongly suggest that the sodium ion rather than the chloride ion is, of the two, the more important factor in the production of edema.

Blackfan and Hamilton [29] (1927) demonstrated a low total base in the serum of children suffering with nephrosis. Albright and Bauer [30] (1929), in their careful study of a patient with nephrosis, made clear the definite relationship between diuresis and the total base excretion in the urine and feces.

Van Slyke [31] (1925) applied the influence of the Donnan equilibrium to the problem of edema. The influence of the nondiffusible

24. Widal, F., and Javal, A.: La cure de dechlororution, Bull. et mém. Soc. méd. d. hôp. de Paris **20**:733, 1903.

25. Blum, L.: Ueber die Rolle von Salzen bei der Entstehung von Oedemen, Verhandl. d. Cong. f. inn. Med. **26**:122, 1909.

26. Blum, L.; Aubel, E., and Hausknecht, R.: Le méchanisme de l'action du chlorure de potassium dans les néphrites hydropignenes, Compt. rend. Soc. de biol. **85**:123, 1921.

27. Magnus-Levy, A.: Alkalichloride und Alkalikarbonate bei Oedemen, Deutsche med. Wchnschr. **46**:594, 1920.

28. Pfeiffer, Emil: Wasserretention durch Natriumsalze, Verhandl. Cong. f. inn. Med. **28**:506, 1911.

29. Blackfan, K. D., and Hamilton, B.: Study of the Inorganic Constituents of the Serum in Children with Acute Nephritis, Bull. Johns Hopkins Hosp. **41**: 322, 1927.

30. Albright, F., and Bauer, W.: Acid Base Balance in Nephritis, J. Clin. Investigation **7**:465, 1929.

31. Van Slyke, D. D.: Factors Influencing the Distribution of Electrolytes, Water and Gases in the Animal Body, Philadelphia, J. B. Lippincott & Company, 1926.

protein ion in causing an unequal distribution of the diffusible ions (the electrolytes of the plasma) will account for about one fifth of the total osmotic pressure of the plasma when in equilibrium with Ringer's solution. If the plasma proteins are reduced, as in nephrosis, this fraction of the osmotic pressure will also be lowered.

The experimental edema which we produced in dogs is the purest form and the most easily controlled type of edema available for study. There is no evidence of the changes in the permeability of the capillaries or in renal function that one might encounter in a patient with the edema of nephritis, nephrosis or cardiac disease, which could alter the result. Therefore, we believed that it would be of value to study the effects of some of the electrolytes on the edema of our animals.

PROCEDURE

Two healthy female dogs, weighing approximately 17 Kg., were selected. Since all the operations were done on unanesthetized dogs, we selected the animals that were least excitable. We avoided the use of depressing drugs as much as possible, as they often cause vomiting and loss of appetite. The dogs were placed on diet A for a control period of one week. Control studies were carried out as described in the protocol of dog 1.

The apparatus for carrying out plasmapheresis (fig. 1) consisted of a Luer-Kaufmann syringe with a no. 18 needle, a 250 cc. pyrex centrifuge bottle and a suction flask with a release pinch cock. The entire apparatus was sterilized for thirty minutes in an Arnold sterilizer. Twenty cubic centimeters of sterile 10 per cent solution of sodium citrate was used for each 250 cc. of blood. The blood was drawn from the femoral artery, as recommended by Barker and Kirk,[5] directly into the centrifuge bottles. These were centrifugated at 1,800 revolutions a minute for from fifteen to twenty-five minutes; the plasma was removed by suction and was replaced by an equal amount of sterile Ringer's solution, and the suspension was filtered through sterile gauze. This solution was reinjected into a vein under pressure. The procedure was carried out twice a day, 500 cc. of blood being withdrawn each time until the plasma proteins fell to about 3 Gm. per hundred cubic centimeters; daily operations thereafter were sufficient to maintain this low level. We constantly observed a slowly increasing anemia, until the hematocrit reading fell to about 30. It usually remained at about this level. If this procedure was repeated over long periods, the dogs became very weak and refused food. It was then necessary to feed protein to them in the form of milk and meat until they regained strength. This always resulted in a rise in plasma protein, with diuresis and loss of edema. The level of the plasma protein was again lowered by the diet low in protein and plasmapheresis, and the studies were continued. With the plasma proteins at a constant low level, the salts, sodium chloride, sodium bicarbonate and potassium chloride were fed during separate periods.

Dog 2.—The dog used in this experiment was a healthy, well nourished, female German shepherd, weighing 16.8 Kg. She was given diet A, which she ate readily. The control studies gave normal results. After a short control period, plasmapheresis was commenced and continued daily for a period of thirty-seven days, with the removal of a minimum of 220 cc. and a maximum of 555 cc. of

plasma daily, making a total of 13,655 cc. during this period. Definite pitting edema of the subcutaneous tissue appeared on the eighteenth day. This was not appreciably increased during a two day period when the intake of water was increased to 1,600 cc. daily. No sodium chloride had been added at the time the edema appeared. On the twenty-first day, the dog was placed on a three day metabolism experiment, receiving 13.9 Gm. of sodium chloride in 1,600 cc. of water by stomach tube daily. The subcutaneous edema increased markedly, ascites appeared, and the dog gained 3 Kg. in weight (table 2). This regimen was repeated for two more days, and the weight increased 2.4 Kg. During the next three days, she was given 1,600 cc. of water alone daily; there was little change in the weight during this period. On the twenty-ninth day, she was started on

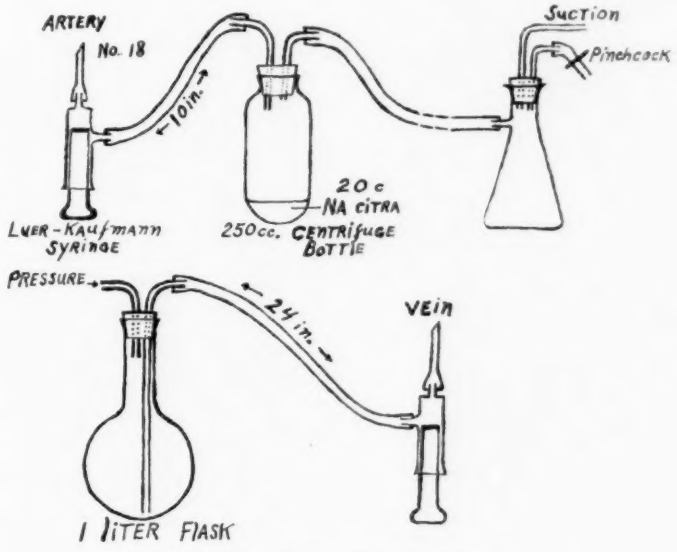

Fig. 1.—Apparatus for plasmapheresis.

a four day metabolism experiment during which she received 19 Gm. of sodium bicarbonate in 1,600 cc. of water daily. She gained 0.3 Kg. during the first day, but from that time on she lost weight. Diuresis set in, and the edema decreased markedly; this continued through the next two days when no bicarbonate was given, and even persisted through a period of three days when 13 Gm. of sodium chloride in 1,600 cc. of water was administered daily. At this time, because of the low hematocrit reading, it was necessary to give the dog a transfusion with 500 cc. of blood from a normal dog. She had become weak and refused diet A, so she was allowed to convalesce for ten days on a diet high in protein.

The second period of plasmapheresis was then commenced and was continued during sixteen days, and a total of 5,810 cc. of plasma was removed. Edema was noticed this time on the seventh day. The amount of edema or the weight failed to increase with the administration of large amounts of water alone. However, when sodium chloride was added, the usual increase in edema and weight was

TABLE 2.—*Results of Studies on Dog 2*

Date, 1939	Wt.	B.P.	Hem.	T.P.	Alb.	Glob.	Fib.	Chol.	B.U. N.	Bl. Cr.	Bl. Cl.	Duration of Metabolism Experiment, Hours	Total Intake of Water, Cc.	Total Output of Urine, Cc.	Ur. Cr.	Ur. Cr.	Ur. N.	Ur. Alb.	Edema	Total Intake of Salt†	Diet	Plasma Carbon Dioxide, per Cent by Volume	Total Amount of Plasma Removed, Cc.	Comment
2/24	16.8	...	51.3	5.5	3.1	2.2	0.2	231	..	...	270	..	ad lib.	.....	...	.....	...	0	0	.....	A	....	0	
2/26	16.1	107	46.6	...	...	...	...	251	7	2.0	290	48	ad lib.	1,750	437	0.43	1.7	0	0	.....	A	....	0	Control period
3/11	15.2	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	..	0	.....	A	....	4,710	
3/14	15.0	...	32.2	2.5	1.2	1.2	0.1	174	..	...	310	72	ad lib.	2,500	320	0.4	2.4	0	0	.....	A	....	5,915	
3/16	.....	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	0	+	.....	A	....	6,730	
3/18	15.2	105	34.1	2.5	1.2	1.2	0.1	135	8	0.6	310	48	3,200	2,800	358	0.9	2.2	0	+	.....	A	....	7,730	
3/19	15.2	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	0	+	.....	A	....	8,280	
3/22	18.2	100	35.5	2.4	1.0	1.2	0.2	151	5	...	330	72	5,500	1,300	217	1.58	...	..	++++	NaCl 35.7 g.	A	....	9,305	
3/24	20.6	110	32.4	2.5	1.1	1.2	0.2	136	..	0.5	350	48	3,200	450	225	1.03	1.9	0	++++	NaCl 27 g.	A	....	9,915	
3/27	20.5	...	35.1	2.5	1.0	1.2	0.3	147	..	0.5	325	72	4,800	2,750	295	2.21	0.7	0	++++	.....	A	....	10,840	Weakness marked
3/31	19.4	...	27.1	2.6	1.2	1.1	0.3	152	..	...	307	96	6,400	3,600	218	1.67	2.9	0	+++	$NaHCO_3$ 76 g.	A	62.6	12,115	
4/ 2	17.1	...	24.7	3.0	0.9	1.8	0.3	189	..	0.6	330	48	3,200	3,800	315	3.31	4.0	0	++	.....	A	39.5	12,775	
4/ 5*	16.1	110	36.0†	2.8	0.9	1.8	0.13	....	5	...	273	72	4,800	4,100	273	6.83	...	0	+	NaCl 38.7 g.	A	....	13,655	
4/16‡	14.7	...	34.7	5.6	2.7	2.5	0.4	142	..	0.9	320	..	ad lib.	.....	...	.....	...	0	0	.....	Prot.§	....	.....	Poor condition; muscular wasting marked
4/20	14.3	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	0	0	.....	A	....	1,170	Condition much improved
4/22	14.5	...	31.8	3.0	1.3	1.5	0.2	...	..	...	...	48	1,600	900	209	0.56	1.4	0	0	.....	A	....	2,260	
4/23	14.5	...	.....	...	...	...	...	...	..	...	...	24	1,500	1,500	...	0.75	2.7	0	+	.....	A	....	2,735	
4/24	14.4	...	36.4	2.88	1.36	1.35	0.17	...	..	0.6	290	..	ad lib.	.....	...	.....	...	0	+	.....	A	....	3,275	
4/26	17.1	...	.....	...	...	...	...	...	..	...	...	48	3,300	350	248	1.2	1.7	0	+++	NaCl 26 g.	A	....	4,010	
4/27	17.5	...	31.0	2.6	1.1	1.4	0.1	...	..	...	350	24	1,600	900	297	1.12	2.1	0	+++	.....	A	43.8	4,285	
4/28	18.2	...	.....	...	...	...	...	...	..	...	280	24	1,600	600	214	0.66	1.8	0	++++	$NaHCO_3$ 19 g.	A	....	4,580	
4/29	18.6	...	34.9	2.9	1.1	1.6	0.2	169	..	...	...	24	1,600	700	259	0.35	1.9	0	++++	$NaHCO_3$ 19 g.	A	....	4,885	
4/30	18.6	...	.....	...	...	...	...	...	..	...	...	24	1,600	1,020	...	0.81	2.6	..	++++	$NaHCO_3$ 19 g.	A	70.2	5,185	Condition fair
5/ 1	18.3	...	33.8	2.5	0.9	1.4	0.24	...	..	0.6	295	24	1,600	1,400	...	0.42	1.7	..	+++	.....	A	69.2	5,485	
5/ 2	18.8	110	.....	...	...	...	...	...	..	...	...	24	1,600	600	200	1.35	1.3	0	++++	NaCl 13 g.	A	....	5,810	
5/ 7#	12.5	...	34.0	3.7	1.5	1.8	0.4	...	..	...	...	..	ad lib.	.....	...	.....	...	..	0	.....	Prot.§	....	.....	Condition fair; muscular wasting marked
5/11	12.6	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	0	0	.....	A	....	1,360	
5/13	12.9	...	34.5	2.5	1.0	1.3	0.2	...	..	...	310	48	1,200	850	181	0.46	1.45	0	+	.....	A	....	2,190	
5/14	12.7	99	36.2	2.9	1.1	1.6	0.2	...	..	0.6	...	24	1,600	1,600	213	1.6	2.7	0	+	.....	A	....	2,435	
5/16	12.5	...	33.0	2.7	1.0	1.3	0.4	180	..	...	290	48	4,400	3,200	192	6.8	1.7	0	+	KCl 29 g.	A	....	3,300	
5/17	12.45	...	.....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	.....	...	0	+	.....	A	....	3,545	
5/19	13.9	...	32.0	2.2	0.9	1.0	0.2	...	..	...	...	48	2,500	730	...	1.78	1.5	0	+++	NaCl 20.7 g.	A	....	4,195	
5/21	15.1	110	30.5	2.5	0.9	1.4	0.2	...	..	...	317	48	3,200	1,550	215	0.62	1.8	0	+++	.....	A	41.4	4,835	
5/23	14.7	...	29.7	2.5	1.0	1.3	0.2	183	..	...	325	48	3,200	2,100	184	2.88	1.5	0	++	Conc. Hcl. 20 cc.	A	35.0	5,460	
5/24	15.5	100	30.2	...	...	...	...	...	7	0.6	325	24	1,630	.....	...	.....	...	0	++++	NaCl 13 g.	A	....	5,790	Killed while it had edema (10 cc. of ether intravenously)

* The sudden rise in the hematocrit was due to the addition of blood cells from 500 cc. of normal dog blood on the fourth.
† The intake of chloride varied from 0.2 to 0.9 Gm. daily except as indicated in this column.
‡ A diet high in protein (consisting of diet A plus 600 Gm. of meat and 500 cc. of milk) was given from the sixth to the sixteenth, inclusively.
§ Diet A; see protocol of dog 1.
# A diet of protein (consisting of diet A plus 200 cc. of milk) was given from the third to the seventh, inclusively.

observed (fig. 2). After this period she was given 19 Gm. of sodium bicarbonate in 1,600 cc. of water daily for three days. At this time there was a definite increase in edema and a gain in weight of 1.1 Kg. (fig. 2 and table 2). A further increase of 0.5 Kg. in weight occurred with the administration of 13.5 Gm. of sodium chloride in 1,600 cc. of water. She was then allowed another short period of convalescence on a diet moderately high in protein (table 2).

The third period of plasmapheresis was then started and continued for eighteen days, and a total of 5,790 cc. of plasma was removed. Edema was noticed on the sixth day. The dog was then given 13.5 Gm. of potassium chloride in 2,200 cc. of water daily for two days. There was no gain in weight and no increase in edema; however, after the administration of 10 Gm. of sodium chloride in 1,200 cc. of water daily for the next two days there was a prompt rise in weight, and

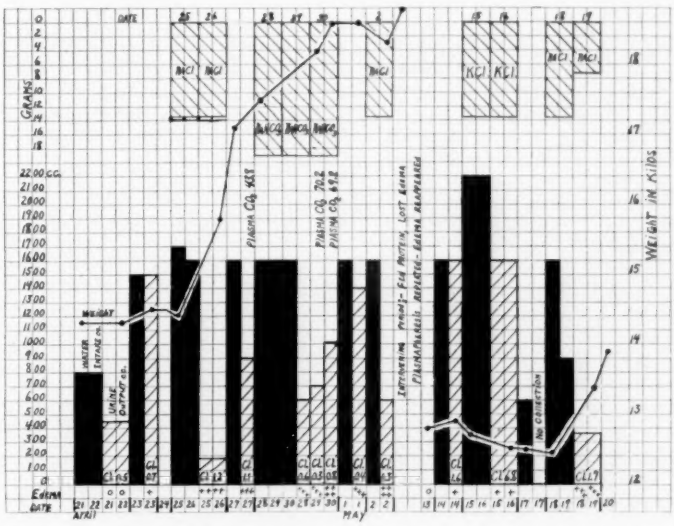

Fig. 2.—Graphic representation of experiments on dog 2. Determinations on the intake and output of fluid are blocked in at the base and those on the salt added to the diet at the top of the chart.

the edema was considerably increased (fig. 2 and table 2). After two days with no added salt in the diet, we attempted to feed her 5 cc. of concentrated hydrochloric acid in 800 cc. of water twice daily for two days. The dog vomited an unknown amount of this acid solution, so quantitative observations were inaccurate; however, a sufficient amount was retained to lower the carbon dioxide-combining power of the plasma from 41 to 35 per cent. There was a slight decrease in edema and she lost 0.4 Kg. Sodium chloride administered during the next day brought about an increase of 0.8 Kg. in weight.

The blood chloride showed little variation during the entire period. The creatinine index remained normal. There were no albumin or casts in the urine at any time. The rectal temperature varied from 101 to 103 F. The systolic blood pressure ranged from 95 to 110 mm. of mercury.

On the ninety-seventh day, while the dog had edema, it was killed by the intravenous injection of 10 cc. of ether, and autopsy was performed. There was marked edema of the subcutaneous tissue, and 1,500 cc. of fluid was removed from the peritoneal cavity. The specific gravity of this fluid was 1.006. The muscles were normal in color and consistency, but were greatly wasted. The left kidney weighed 39 Gm., the right, 41 Gm. The capsules stripped easily, leaving a smooth glistening surface which contained many small white flecks but no scars. The ratio of the width of the cortex to the medulla was normal. The urine from the bladder showed no albumin, and the sediment showed only an occasional white blood cell. The other organs were normal in appearance and weight.

The sections of the kidney stained with eosin-methylene blue and scarlet red showed a fatty degeneration of the collecting tubules, of the distal convoluted tubules and to a very slight extent of the proximal convoluted tubules. In many instances the fat was at the base of the cell and about the nucleus, and at times it filled the entire cell. The nuclei were well preserved, and a hyperchromatic or pyknotic nucleus was rarely seen. The remainder of the kidney cells showed only edema. The loops of Henle and the proximal convoluted tubules on the whole were well preserved. The tubules contained only an occasional hyaline cast. The glomeruli showed no lesions in the form of a fibrosis, epithelial proliferation or infiltration with inflammatory cells. There was no increase in the interstitial tissue.

Dog 3.—Dog 3 was a well nourished, healthy, female shepherd dog, weighing 17.1 Kg. This dog was given diet A during the periods of plasmapheresis and usually consumed all of it. All of the control studies were normal (table 3). The first period of plasmapheresis was then commenced and continued for twenty-two days, with the removal of from 195 to 515 cc. of plasma daily, making a total of 7,095 cc. Edema was first noticed on the seventeenth day. A three day metabolism experiment with the addition of 1,600 cc. of water daily by stomach tube failed to increase the edema or the weight. However, during the next two days, 13.5 Gm. of sodium chloride was added with the same amount of water each day; the edema increased markedly, and there was a gain in weight of 1.9 Kg. An attempt to give the dog sodium bicarbonate failed because of increasing weakness and loss of appetite. At this time plasmapheresis was discontinued and she was placed on a diet high in protein for three days; she regained her usual good health.

The second period of plasmapheresis was started and continued for twelve days, with a total removal of 5,200 cc. of plasma. Edema was first noticed on the seventh day. The dog again gained in weight and edema on sodium chloride given as before, and she maintained the same condition when given water alone for one day.

The dog was then placed on a three day experiment, receiving 19 Gm. of sodium bicarbonate in 1,600 cc. of water daily. This time she did not become ill, and there was a gain in weight of 0.9 Kg., with a definite increase in edema. During the experiment with sodium bicarbonate the dog was given a transfusion of blood because of the accidental clotting of her own blood during the centrifugation. This transfusion was performed with the blood cells from 500 cc. of normal dog's blood suspended in saline. The experiment was followed with 1,600 cc. of water, and this resulted in a further increase in edema and a gain of 0.2 Kg. This concluded the second period of plasmapheresis. Because of the dog's increasing weakness, 200 cc. of milk was added to diet A for six days.

The third period was then commenced and continued for fourteen days with a total removal of 4,605 cc. of plasma. Edema appeared on the seventh day. Again

TABLE 3.—*Results of Studies on Dog 3*

Date	Wt.	Hem.	T.P.	Alb.	Glob.	Fib.	Chol.	B.U. N.	Bl. Cr.	Bl. Cl.	Duration of Metabolism Experiment, Hours	Total Intake of Water, Cc.	Total Output of Urine, Cc.	Ur. Cr.	Ur. Cl.	Ur. N.	Ur. Alb.	Edema	Total Intake of Salt*	Diet	Total Amount of Plasma Removed, Cc.	Comment
3/23/30	17.1	53.6	5.3	3.2	1.9	0.2	189	6	...	...	..	ad lib.	.....	...	....	...	..	..	.....	A	0	
3/26/30	16.9	52.7	6.2	3.3	2.1	0.8	...	..	1.3	275	72	2,400	2,100	437	0.63	2.3	0	0	.....	A	0	Control period
4/ 3/30	16.1	48.5	3.2	1.9	1.0	0.3	149	..	...	300	..	ad lib.	.....	...	....	...	0	0	.....	A	1,870	
4/ 6/30	15.8	45.5	3.2	1.6	1.1	0.5	156	..	1.4	300	72	ad lib.	1,700	391	0.28	3.0	0	0	.....	A	3,385	
4/ 9/30	15.75	40.9	2.75	1.3	1.1	0.32	...	..	...	285	..	ad lib.	.....	...	....	...	0	0	.....	A	4,780	
4/12/30	15.3	35.1	2.13	1.3	0.7	0.16	146	..	...	...	72	4,800	3,850	240	0.64	2.9	0	+	.....	A	6,520	
4/14/30	17.2	34.9	2.34	1.0	1.1	0.14	152	..	...	325	48	3,200	710	253	1.63	2.4	..	+++	NaCl 26 g.	A	7,095	Condition poor; nausea vomiting
4/20/30†	14.5	30.8	5.5	3.1	1.6	0.8	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	Prot.*	.....	Condition fair
4/23/30	14.7	28.6	3.5	2.0	1.2	0.3	...	..	0.7	300	24	1,500	1,400	261	0.98	5.1	0	0	.....	A	1,710	
4/24/30	14.8	33.7	3.12	1.8	1.1	0.21	...	..	0.6	300	..	ad lib.	.....	...	....	...	..	+	.....	A	2,320	
4/26/30	16.9	31.8	2.4	1.3	0.9	0.2	...	..	...	340	48	3,200	490	203	2.02	3.1	0	+++	NaCl 26 g.	A	3,100	
4/27/30	16.8	....	...	...	...	...	...	..	...	...	24	1,600	1,600	260	2.1	2.6	0	+++	.....	A	3,490	Carbon dioxide per cent by volume, 49.4
4/28/30	17.5	....	...	...	...	...	...	..	...	...	24	1,600	720	...	0.9	3.1	..	++++	NaHCO3 19 g.	A	4,080	Carbon dioxide per cent by volume, 69.2
4/29/30‡	17.8	40.2	2.5	1.0	1.4	0.15	174	..	...	280	24	1,600	950	...	0.9	3.0	0	++++	NaHCO3 19 g.	A	4,334	Carbon dioxide per cent by volume, 68.3
4/30/30	17.8	....	...	...	...	...	...	..	...	...	24	1,600	500	...	0.67	1.6	0	++++	NaHCO3 19 g.	A	4,620	Condition improved
5/ 1/30	17.3	35.5	2.5	1.0	1.3	0.26	...	..	0.6	300	24	1,600	1,600	...	0.4	2.4	0	+++	.....	A	4,900	
5/ 2/30	17.5	....	...	...	...	...	...	..	...	...	24	1,600	1,200	...	4.5	2.2	0	++++	NaCl 13.5 g.	A	5,200	
5/ 8/30§	12.8	30.3	3.9	1.6	2.2	0.12	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	Prot.*	.....	Muscular wasting apparent
5/11/30	12.75	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	0	0	.....	A	1,180	
5/13/30	12.9	34.7	2.7	0.9	1.7	0.1	...	..	...	315	48	1,400	1,100	181	0.63	1.5	0	0	.....	A	2,345	
5/14/30	12.7	38.2	2.9	1.2	1.3	0.23	...	..	0.6	300	24	2,200	1,590	212	1.9	5.5	0	+	.....	A	2,640	
5/16/30	12.1	31.2	2.4	1.2	1.1	0.1	174	..	...	...	48	4,400	3,330	196	8.3	1.4	0	+	kCl 26 g.	A	3,295	
5/17/30	12.0	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	..	+	.....	A	3,705	
5/19/30	13.5	30.5	2.0	0.9	0.9	0.15	...	..	...	...	48	2,500	330	...	0.57	0.7	0	+++	NaCl 20 g.	A	4,265	
5/20/30	14.1	30.1	2.1	0.8	1.1	0.2	...	..	...	352	24	1,600	.....	...	....	...	0	+++	.....	A	4,605	Condition poor
5/23/30	14.1	....	...	...	...	...	...	..	...	...	..	ad lib.	.....	...	....	...	0	+++	.....	A	4,920	
5/26/30	11.3	35.7	2.3	1.0	1.2	0.1	202	11	0.7	305	72	2,100	3,700	308	4.87	...	0	0	.....	Prot.	.....	Condition improved; killed 5/27/30 (10 cc. of ether intravenously)

* The intake of chloride varied from 0.2 to 0.9 Gm. daily, except as indicated in the column headed "total intake of salt." This was due to the excess of chloride in the Ringer's solution over the average plasma chloride.

† A diet of protein (consisting of diet A plus 600 Gm. of meat and 500 cc. of milk) was given from the eighteenth to the twentieth, inclusively.

‡ The rise in the hematocrit was due to the addition of blood cells from 500 cc. of normal dog blood.

§ A diet of protein (consisting of diet A plus 200 cc. of milk) was given from the third to the eighth, inclusively.

the dog failed to gain weight or the edema to increase during a one day experiment with 2,200 cc. of water. This was followed by a two day experiment, during which we administered 13.5 Gm. of potassium chloride in 2,200 cc. of water daily. There was no increase in edema or in weight; in fact, there was a loss of 0.6 Kg. The next day 1,600 cc. of water alone was given, and there was no change in weight, but an experiment on the following two days, consisting of a daily intake of 10.3 Gm. of sodium chloride in 1,300 cc. of water, caused a marked increase in edema and a gain of 1.5 Kg. We attempted to administer dilute hydrochloric acid, but this dog vomited all of the solution. We felt that it might be interesting to kill one dog while she had massive edema and the other when there was no edema, in order to see if there was any detectable change in the fixed tissue. Therefore, dog 3 was given a diet high in protein; the edema disappeared, and she was killed on the ninetieth day of the experiment by the intravenous injection of 10 cc. of ether. Frequent urinalyses during the experimental period and urinalysis at autopsy revealed no albumin or casts. These results were substantiated by the failure to find casts in the renal tubules (see autopsy). The creatinine index remained normal throughout. The rectal temperatures showed no variation from the normal.

No subcutaneous edema or excess of fluid in the cavities of the body were found at autopsy. The left kidney weighed 62.5 Gm., and the right, 62 Gm. The capsules stripped readily, leaving a smooth, glistening surface with no gross abnormality. The urine of the bladder showed no albumin, and the sediment contained only an occasional white blood cell. All of the other organs were normal in appearance and weight.

The sections of the kidney stained with eosin-methylene blue and scarlet red revealed a rather marked fatty degeneration of the collecting and distal convoluted tubules, characterized by vacuoles of fat of varying sizes, which at times completely filled the cells and caused some enlargement of the cell, so that the lumen of the tubule was narrowed. The degree of involvement in this kidney was greater than that in dog 2 and more nearly approached the degree in involvement found in dog 1. It differed from the latter, however, in that the fat was limited to the distal and convoluted tubules with almost no fat in the proximal convoluted tubules or in the loops of Henle. The glomeruli were normal in appearance; there was no proliferation of the capsule or tuft epithelium, fibrosis or infiltration with inflammatory cells. There was no hyaline membrane with fat contained around the glomeruli. There was no increase in the interstitial tissue, and the occasional area of lymphocytic infiltration was so small that it was hardly worth consideration.

RESULTS AND COMMENT

The Influence of Sodium Chloride on Experimental Edema.—In the dogs edema did not develop until the plasma proteins fell to about 3 Gm. and the albumin to about 1 Gm. per hundred cubic centimeters. At this level, without the use of excessive amounts of water or sodium chloride, dogs 2 and 3 showed definite pitting edema. The use of large amounts of distilled water (1,600 cc.) by stomach tube did not increase the edema or the weight appreciably, but when 13 Gm. of sodium chloride per day was added to the water, massive edema appeared, accompanied by a rapid increase in weight. There were ascites and marked pitting edema of the skin, especially where there were loose folds of flesh and particularly in the hind legs. It seems that, given

a low plasma protein, the amount of edema is determined by the amount of sodium chloride administered. This is demonstrated in figure 2. We wish to make clear that a constant low level of plasma proteins was maintained while the influence of the various salts was studied.

The Influence of Sodium Bicarbonate on Experimental Edema.—We have not included in the following discussion the results of the first experiment with sodium bicarbonate, which is described in the protocol of dog 2, for reasons given in section 2 of the next division of the paper.

The administration of sodium bicarbonate gave the same sort of response as did sodium chloride but to a lesser degree, in spite of the use of larger amounts of the bicarbonate, i. e., 19 Gm. per day. The amount used was sufficient to change the plasma carbon dioxide-combining power from 44 to 70 per cent in dog 2, and from 49 to 69 per cent in dog 3, during the first twenty-four hours. During the three days that this salt was given, the weight of dog 2 increased 1.1 Kg., and the weight of dog 3 increased 1 Kg. (fig. 2 and tables 2 and 3). We were unable to continue giving sodium bicarbonate to these dogs, as they were very weak and refused food. This result is interesting in view of the enthusiasm for the use of alkali as a diuretic which has been renewed recently by Osman [32] and also by Bennett.[33] However, it is worthy of note that half of the alkaline salts given by these authors were potassium salts. After our experiment was concluded the dogs were given meat and milk; the edema promptly disappeared, and they regained their strength.

The Influence of Potassium Chloride on Experimental Edema.—After the level of the plasma protein was lowered by plasmapheresis and slight edema was established in both dogs, 13.5 Gm. of potassium chloride was administered daily for two days. This was given in water sufficient to make a nearly isotonic solution (1,600 cc.). During this two day period dog 2, instead of gaining weight, lost 0.2 Kg., and dog 3 lost 0.6 Kg. (fig. 2 and tables 2 and 3). In order to ascertain whether or not this loss of weight was due to the onset of a spontaneous diuresis, we administered sodium chloride, and both dogs showed a typical rise in weight, and the edema was increased. This failure to increase edema with the administration of potassium chloride was also observed with dog 1.

The Influence of Hydrochloric Acid on Experimental Edema.—The administration of a dilute solution of hydrochloric acid to these dogs induced vomiting, so we were unable to make quantitative obser-

32. Osman, A. A.: Studies in Bright's Disease, Guy's Hosp. Rep. **77**:386, 1927.

33. Bennett, T. I.: Nephritis: Its Problems and Treatment, London, Oxford University Press, 1929.

vations. However, a sufficient amount was retained by dog 2 to lower the carbon dioxide-combining power of the plasma from 41 to 35 per cent, and this was accompanied by a loss of 0.2 Kg.

The output of creatinine was studied as a check on the collection of urine and as an index of renal function. There was a gradual fall in both the creatinine of the blood and that of the urine, so that the index $\frac{\text{urine creatinine}}{\text{blood creatinine}}$ showed no fall. The chloride metabolism was studied. We believe that a study of the total base or the sodium metabolism would be of more value than one of the chloride, but we felt that in these preliminary experiments it would be better to overcome some of the technical difficulties accompanying the production of edema before attacking the problem of total base determinations in the serum, urine and feces of dogs. We regret that the number of experiments reported here is necessarily small, but we have tried to avoid drawing conclusions that are too broad, except when our conclusions were strengthened by previous good experimentation of others.

OTHER INTERESTING PHASES OF EDEMA

1. *Relation of Edema to the Chloride Excreting Power of the Kidney.*—In the past many workers expressed the belief that the rôle of sodium chloride in the production of edema is due to the failure of the kidney to excrete either the sodium or the chloride ion. The result of this is a retention of salt in the tissues of the body; this salt holds water with it, hence there is edema. However, at present most of the investigators who are interested in the problems of edema believe that the kidney has little to do with the retention of salt, particularly in the edemas originating in low plasma protein. It is more likely that the lowered osmotic pressure of the plasma proteins allows fluid to escape into the tissues, and this fluid carries salt with it which is retained there, so that the kidney is never offered the major portion of the salt for excretion. We have evidence that during the edematous periods, the kidneys of our dogs were capable of excreting chloride normally. Unfortunately we are unable to offer the same evidence for the sodium ion as the excretion of sodium was not studied.

The function of the kidneys of our dogs was normal by the usual methods of study. The intake of chloride, except during the periods when measured amounts were added, ranged from 0.2 to 0.8 Gm. per day. The output of chloride was roughly equal to the intake even during the periods of massive edema unless the weight was increasing. If the dogs were gaining weight, the output of chloride would obviously be less than the intake, because the chloride would be retained in the tissues along with the base. Even while the dogs were rapidly gaining weight and edema was increasing as a result of feeding large amounts of sodium chloride, the kidneys were excreting much more chloride than they

normally excreted. For example, dog 2 was given a total of 19.6 Gm. of chloride (36 Gm. of sodium chloride) during three days while the plasma proteins were low, and she excreted 4.7 Gm. of chloride. During this period she gained 3 Kg. When the plasma proteins were higher, she was given the same amount of chloride and excreted all of it in the three days (table 1). The same result was observed in dog 3. All of the chloride was also excreted when potassium chloride was given. This ability to excrete large amounts of chloride during the edematous period is certainly evidence against any defect in the chloride excreting power of the kidney.

2. *Spontaneous Diuresis in the Edema of Low Plasma Proteins.*—There has been a hesitancy to accept low plasma proteins as the main factor in the causation of this type of edema, because certain patients who have low plasma proteins have no edema; also during the course of observation of patients with nephrosis who show edema, one will at times observe a spontaneous diuresis with the loss of edema in spite of persistence of low plasma proteins and no change in the albumin-globulin ratio. Moore and Van Slyke [9] reported such a case and called attention to some "unknown factor" which may cause this. Leiter,[4] as well as Barker and Kirk,[5] observed a tendency for the edema in their dogs to disappear as the experiments were prolonged, in spite of the maintenance of low levels of plasma protein.

This type of spontaneous diuresis probably occurred in one of our dogs (dog 2) while we were administering sodium bicarbonate, and was the cause of some confusion. This dog had had extensive edema for fifteen days when we gave her 19 Gm. of sodium bicarbonate daily during four days. She gained 0.3 Kg. the first day and then began to lose weight rapidly; the edema decreased, and there was a marked diuresis. We then gave her 13.5 Gm. of sodium chloride daily for three days, which in every other experiment resulted in a rapid rise in weight, but at this time the diuresis persisted and the edema practically disappeared. A chemical analysis of the blood revealed no cause for this; the total plasma proteins were 2.8 Gm. and the albumin 0.9 Gm. per hundred cubic centimeters. The carbon dioxide had fallen to the original level of 39 per cent, and the results of other studies were normal. When the same amount of sodium bicarbonate was given to this dog after it had been edematous for only a short period, she gained weight in the same way in which dog 3 did. Jansen,[34] Magnus-Levy [27] and others always observed an increase in the edema of their patients after the administration of sodium bicarbonate. For these reasons we believe that a spontaneous diuresis occurred during this period of study, and that probably the sodium bicarbonate had little

34. Jansen, W. H.: Der Oedemkrankheit, Deutsches Arch. f. klin. Med. **131**: 144, 1919.

or nothing to do with it. The onset of such a spontaneous diuresis during the study of diuretic drugs may be the cause of considerable confusion and may be a reason for false ideas concerning their efficacy.

3. *Relation of Experimental Edema Produced by Lowering the Plasma Proteins to Nephrosis.*—Barker and Kirk drew attention to the similarity between the edema of dogs produced through plasmapheresis and the clinical syndrome of nephrosis. We have been able to confirm their observations. However, we were not able to find glomerular lesions or albumin or casts in the urine, but we invariably found an excessive amount of lipoid deposit in the renal tubules. In order to control this side of our problem, we made fat stains from the fresh kidney tissue of two dogs, one young and the other full grown. Both showed a few fat droplets in the renal tubules, but the amount present was not comparable to that found in our experimental animals. Glomerular lesions of various sorts were not uncommon in these so-called normal dogs.

The picture in the dog with edema produced through deprivation of protein was identical with that in the dogs in which edema had been produced by plasmapheresis. We feel that this condition is not nephrosis in the dog, but we regard the syndrome observed in all of these dogs as a specific result of the deprivation of protein. We may go even further and state that perhaps certain changes in patients with nephrosis, namely, edema, low plasma protein with reversed albumin-globulin ratio, fatty degeneration of the renal tubules, low basal metabolic rate and possibly cholesterolemia, is the result of deprivation of protein, which in this instance is the result of loss of protein through the kidney, often augmented by a diet low in protein.

4. *The Reasons for the Reversal of the Albumin-Globulin Ratio.*—It has been suggested that the reversal in the albumin-globulin ratio in nephrosis in human beings is due to the selective loss of the smaller albumin molecule through the kidney.[35] As early as 1916, Kerr, Hurwitz and Whipple [36] observed a reversal in the albumin-globulin ratio in their excellent studies on plasmapheresis; in such experiments, there is no selective loss of albumin. They gave three possible reasons for this phenomenon, namely, the fact that shock or infection occurred, that globulin is regenerated faster than albumin in serum, or that during the forced regeneration the precipitation values of the proteins may vary from normal. We can eliminate the first possibility, for there was neither shock nor infection in any of our animals. We were unable to find any work which showed that the precipitation factors of the protein

35. Cowie, D. M.; Jarvis, K. M., and Cooperstock, M.: Metabolism Studies in Nephrosis, Am. J. Dis. Child. **40**:465 (Sept.) 1930.

36. Kerr, W. J.; Hurwitz, S. H., and Whipple, G. H.: Regeneration of Blood Serum Proteins, Am. J. Physiol. **47**:356, 1918.

were changed. Probably the simplest explanation is that the body is able to regenerate globulin from the tissues more readily under these conditions than it does albumin.

5. *The Edema Fluid in Experimental Edema.*—The edema fluid was studied in dog 1, in which edema was produced by a diet, and in dog 2, in which edema was produced by diet and plasmapheresis (table 4). The figures for protein and chloride are essentially the same for the two dogs. The protein is a little higher than is usual in this type of edema fluid, possibly because of contamination with small traces of blood during collection. The figure for chloride agrees with that found in the edema fluid from patients with nephrosis and in normal spinal fluid. It is interesting to note that during the period of administration of sodium bicarbonate, the level of chloride of the edema fluid fell from 450 to 375 mg. per hundred cubic centimeters.

TABLE 4.—*Summary of Determinations Made on Edema Fluid*

Dog	Date	T.P. of Edema Fluid, per Cent	Cl. of Edema Fluid, Mg. per 100 Cc.
1	6/ 6/30	0.21	420
2	3/22/30	0.19	420
	3/25/30	0.19	450
	3/29/30*	0.13	375

* Determinations on this date were made after the administration of sodium bicarbonate.

CONCLUSION

1. Edema was produced in a dog by a diet low in protein but adequate in carbohydrate and fat. The chemical, pathologic and clinical observations made on this animal were essentially the same as the observations made on those in which edema had been produced by selective removal of plasma proteins (plasmapheresis).

2. The chemical, pathologic and clinical determination made on all of the dogs were similar to those found for dogs in which edema was produced through an inadequacy of protein in the diet.

3. Sodium chloride and sodium bicarbonate increased the edema of dogs with a constant low level of plasma protein, while potassium chloride did not. This is further evidence that the sodium ion has a greater influence on edema than the chloride ion. It seems probable that given a low plasma protein, the amount of edema is determined by the quantity of the intake of the sodium ion.

4. Evidence is presented to show that the power of the kidney for excreting chloride is normal in edema produced experimentally with a low plasma protein.

Dr. J. P. O'Hare supervised us in this work and Miss Margaret Driscoll gave technical aid.

INSULIN

SOME EFFECTS ON NORMAL RABBITS PROTECTED FROM HYPOGLYCEMIA BY INGESTION OF DEXTROSE *

ROBERT F. LOEB, M.D.

EMILY GUILD NICHOLS

AND

BERYL H. PAIGE

NEW YORK

In many clinics, the technic of insulin therapy as applied to the treatment for diabetic acidosis has undergone a series of changes since the introduction of insulin in 1922. At the Presbyterian Hospital, as elsewhere, brilliant therapeutic results were obtained in the early days of the use of insulin by the administration of relatively small doses of the hormone in conjunction with the supportive measures employed in the pre-insulin era. When large quantities of insulin became available for clinical use, massive doses were employed on the assumption that they would bring about more rapid alleviation of the ketosis by augmenting the oxidation of dextrose. The results of this type of therapy were, however, frankly disappointing, in part at least, because too much emphasis was placed on disturbances of carbohydrate oxidation and not enough on the significant consequences of severe ketosis, namely, loss of fixed base, dehydration and collapse. In recent years, patients in this clinic have again been treated with small doses of insulin, more attention has been paid to the dangers of dehydration, and the results have again been most gratifying.

In spite of this improvement in the methods of treatment for diabetic acidosis, a number of patients die, and death not infrequently occurs some hours after the diabetes and ketosis have been controlled. The cause of the fatal outcome in these cases has frequently been ascribed to a deleterious effect of insulin. This idea has led to numerous clinical and laboratory studies to determine possible toxic effects of insulin on

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* From the Departments of Medicine and Pathology of the College of Physicians and Surgeons of Columbia University and the Presbyterian Hospital.

the heart, circulation, nervous system, muscles and suprarenal glands.[1] Most of these investigations have been concerned with effects of the hormone in the presence of severe hypoglycemia. In view of the fact that this state is rarely encountered in the treatment for diabetic acidosis, it seemed desirable to study the comparative effects of small and massive doses of commercial and of crystalline insulin in the normal animal, protected against hypoglycemic shock by means of adequate amounts of dextrose.

In this work an attempt has been made: (1) to determine the possible general toxic effects of large doses of insulin in contrast to small ones; (2) to compare the actual, as well as the relative, effects of small and large doses of the hormone on the hyperglycemia resulting from continuous absorption of dextrose; (3) to observe the effects on the glycogen storage in the liver, and (4) to note any possible histologic changes produced in the adrenal glands and liver. It was hoped that such a study might furnish evidence that might be of value in determining the relative merits of small and of massive doses of insulin.

EXPERIMENTAL WORK

Two main groups of studies were made on healthy adult rabbits. One group of animals was fed on a diet of oats and cabbage up to the time of the experiment, to insure the presence of large stores of glycogen. A second group was deprived of food for forty-eight hours, so that the content of glycogen at the beginning of the experiment might be at a minimum.

After initial determinations of blood sugar, the animals were given 12 Gm. of dextrose (in 50 per cent solution) per kilogram of body weight by means of a stomach tube. The feedings of dextrose were immediately followed by intravenous injections of insulin, and samples of blood for sugar determinations were taken at hourly intervals for a period of four hours. At the end of this time a second and equal dose of dextrose was given, again followed by insulin, and the blood sugar was determined for the next three hours at hourly intervals. Seven and one-half hours after the initial dextrose feeding, the animals were killed by a sudden blow, and parts of the liver were immediately removed for determinations of glycogen.

In each group of animals, one series received no insulin, another received 7 clinical units of insulin per kilogram (i. e., "small" doses) and a third series received 75 units of insulin per kilogram (i. e., "massive" doses). Crystalline insulin was prepared from commercial insulin furnished us by the E. R. Squibb Company. The preparation of the crystals was carried on according to the method

1. Edwards, D. J., and Page, I. H.: Am. J. Physiol. **69**:177, 1924. Hornung, S.: Compt. rend. Soc. de biol. **97**:1500, 1927. Labbé, M., and Boulin, R.: Médicine **9**:746, 1928. Sundberg, C. G.: Compt. rend. Soc. de biol. **89**:807, 1923. Jung, L., and Auger, L.: Compt. rend. Soc. de biol. **101**:821, 1929. Räihä, C. E.: Skandinav. Arch. f. Physiol. **56**:243, 1929. Baur, H.: Beitr. z. path. Anat. u. z. allg. Path. **83**:1, 1929. Hetenyi, G.: Wien. Arch. f. inn. Med. **13**:95, 1926. Reinwein, H.: Deutsche med. Wchnschr. **55**:951, 1929. Doisy, E. A., and Weber, C. J.: J. Biol. Chem. **58**:721, 1924.

of Prof. John Abel, who furnished us with sample crystals. The crystals obtained by us were identical with those described by Abel, and they assayed about 25 units per milligram.

Blood sugar was determined by the method of Hagedorn and Jensen[2] on samples obtained from the central artery of the ear. Determinations of glycogen were made according to a modification of the Pflüger method, and no estimation was made of reducing substances other than dextrose as the quantities of glycogen were, in most cases, large enough to eliminate great errors from this source. Only the comparative values for glycogen in various groups of animals seemed of interest. All analytic procedures were carried out in duplicate.

Animals in which any appreciable diarrhea occurred during the experiment were discarded. Loss of insulin through renal excretion was slight, as most of the animals voided but little. Furthermore, in one instance, 20 per cent of the urine excreted by a rabbit in seven hours after receiving 444 units of insulin intravenously was injected subcutaneously into a second rabbit and failed to cause convulsions in nine hours.

For the histologic studies, both adrenal glands and the liver were removed as soon as possible after death. The organs were weighed, as were the animals after the removal of the stomach and intestinal tract. Comparisons of the weights of the adrenals and liver in relation to body weight were noted in the various groups of experiments performed. The adrenal glands were sectioned, part being placed in "Müller-formol" and the remainder in a formaldehyde solution (solution of formaldehyde, U. S. P. 1, water to make 10). Blocks of tissue fixed in "Müller-formol" were transferred to Müller's solution, washed in running water, dehydrated in alcohol and embedded in paraffin, and several sets of serial sections were stained with hematoxylin and eosin. Frozen sections from the tissue fixed in formaldehyde were stained with scharlach R. Since a comparison of the sections of the adrenal glands of well-fed and of starved control animals to which no insulin was administered showed great variation in the widths of the cortex and of the medulla in individual animals, no accurate measurements were made, the widths of layers as seen in the hematoxylin-eosin preparation being merely compared and recorded as "wide," "average" and "narrow." From the hematoxylin-eosin sections the intensity of the chromaffin staining was also determined, and a search was made for any structural changes that might be present. From the frozen section stained with scharlach R the relative amounts of lipoid in the different glands were determined.

Blocks of liver tissue were fixed in Zenker's solution, 10 per cent formaldehyde and 95 per cent alcohol. From the blocks fixed in Zenker's solution paraffin sections were obtained and stained with hematoxylin and eosin. Tissue fixed in formaldehyde was used for frozen sections which were stained with scharlach R. From the blocks fixed in alcohol, celloidin sections were stained with Best's carmine to demonstrate glycogen.

RESULTS

Blood Sugar Curves.—The marked fluctuations in blood sugar curves in the animals receiving identical treatment may be observed in any one or in all of the tables. The variations are so great that mathematical handling of the data is impossible, and consequently the evaluation of results is difficult.

2. Hagedorn, H. C., and Jensen, B. N.: Biochem. Ztschr. **128**:185 and 207, 1922.

It was found in a series of fourteen well-fed rabbits receiving "small" doses of insulin after the ingestion of dextrose that there resulted a greater depression of the average blood sugar curve than in a group of eighteen animals receiving "massive" doses of the hormone (tables 1, 2 and 3 and chart 1). In view of the enormous differences in the individual reaction of the animals, it does not seem justifiable to draw conclusions beyond stating that there was a "tendency" for the blood sugar levels of well-fed rabbits to be depressed more by "small" doses of insulin than by "massive" ones after the ingestion of dextrose.

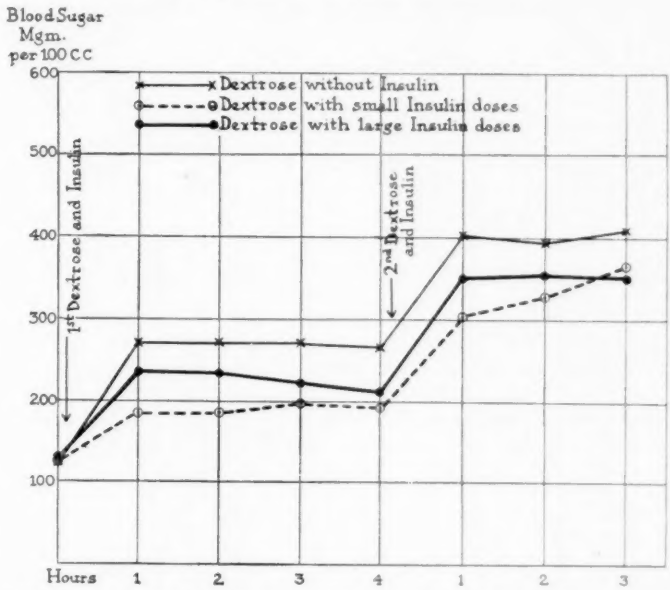

Chart 1.—Average blood sugar curves of well-fed rabbits that received: (1) dextrose without insulin, (2) dextrose with "small" doses of insulin and (3) dextrose with "massive" doses of insulin. A number of results which were similar to those presented have been omitted from chart 1 because a less reliable method was used for the determination of the blood sugar in the first experiments.

In the group of animals starved for forty-eight hours before the administration of dextrose and insulin, no constant or consistent difference was noted in the response to "small" doses of insulin in contrast to "massive" ones (tables 4, 5 and 6).

Experiments carried on with crystalline insulin (tables 7 and 8) showed no definite differences in the behavior of the blood sugar curves after "small" as compared with "massive" doses of insulin, but the number of animals used was too small to be of significance.

TABLE 1.—*Well-Fed Rabbits That Received No Insulin After Feeding of Dextrose**

Date	Rabbit Number	Blood Sugar After First Dextrose: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose, per Cent	Comment
12/26/28	14	131	313	342	332	313	342	426	400	12.4	
12/29/28	17	125	260	206	230	266	...	...	...	....	Rabbit weak; convulsed and died 1 hour after second administration of dextrose
1/ 2/29	18	125	247	284	300	241	304	278	282	13.1	
1/ 3/29	19	138	245	238	238	253	306	350	422	14.9	
1/ 4/29	20	98	417†	428	428	458	508	483	483	13.1	Slight diarrhea
1/ 9/29	21	122	260	282	282	296	249	313	345	14.3	
1/11/29	22	130	312	307	295	215	570	709	746	....	Rabbit died before liver was removed; was weak and drowsy
8/16/29	72	127	263	237	236	232	624	...	...	....	Grew progressively weaker and died
8/17/29	73	129	243	249	221	238	...	...	...	....	Neck broken and animal killed at time of second feeding of dextrose
8/17/29	74	127	193	208	236	268	506	...	...	....	Rabbit grew weak and died
8/19/29	75	136	232	199	188	188	206	208	191	11.5	
Average.....		126	271†	271	271	269	402	395	410	13.2	

* A number of results which were similar to those presented have been omitted from tables 1, 2 and 3 because a less reliable method was used for the determination of the blood sugar in the first experiments.

† Approximate value.

TABLE 2.—*Well-Fed Rabbits Receiving Small Doses of Insulin (Seven Units Per Kilogram) After Each Dextrose Feeding*

Date	Rabbit Number	Blood Sugar After First Dextrose and Insulin: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose and Insulin: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose and Insulin, per Cent	Comment
11/27/28	2	117	166	157	149	147	235	204	204	10.1	Slight diarrhea at end of experiment
12/ 3/28	4	138	108	129	133	135	151	167	195	9.7	Slight diarrhea at end of experiment
12/ 5/28	6	121	122	115	111	126	184	212	268	8.7	
12/11/28	8	123	153	212	210	216	267	271	285	12.8	
12/14/28	10	131	219	228	232	213	280	280	284	7.6	Some diarrhea; liver fibrotic; somewhat dyspneic
12/18/28	12	121	163	181	174	202	221	243	283	5.8	A little dyspneic
8/ 2/29	58	124	207	244	261	248	447	485	728	10.6	
8/ 3/29	60	129	163	131	145	141	287	...	...	....	Rabbit grew restless and weak and then died; slight diarrhea; autopsy negative
8/ 5/29	61	131	253	294	302	278	575	506	510	13.8	
8/ 6/29	63	119	195	198	195	157	336	452	...	....	Rabbit became weak and died; slight diarrhea toward end
8/ 7/29	65	138	226	236	243	228	358	430	480	12.7	
8/ 9/29	68	150	245	208	228	219	318	372	398	11.2	
Average.....		129	185	186	198	193	305	329	364	10.3	

TABLE 3.—*Well-Fed Rabbits That Received Massive Doses of Insulin (Seventy-Five Units Per Kilogram) After Each Feeding of Dextrose*

Date	Rabbit Number	Blood Sugar After First Dextrose and Insulin: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose and Insulin: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose and Insulin, per Cent	Comment
11/26/28	1	124	202	175	133	118	288	...	221	8.9	
12/ 1/28	3	131	152	143	151	147	300	272	311	8.4	Rabbit weak and dyspneic at end of experiment
12/ 4/28	5	128	331	316	306	304	477	466	392	13.0	Hind legs somewhat weak
12/10/28	7	122	160	218	243	230	354	271	383	6.0	Somewhat dyspneic toward end of experiment
12/12/28	9	139	342	362	356	327	431	449	420	12.5	Somewhat dyspneic toward end of experiment
12/17/28	11	122	196	143	136	133	244	222	216	8.8	
12/19/28	13	132	190	262	293	328	452	430	416	7.2	Rabbit somewhat dyspneic throughout experiment
8/ 2/29	57	110	171	158	153	137	....	....	....	....	Weak after second administration of dextrose and insulin
8/ 3/29	59	134	241	220	211	206	302	296	328	12.0	
8/ 6/29	62	141	253	244	211	211	322	344	364	12.2	Rather drowsy and had slight diarrhea at end of experiment
8/ 7/29	64	132	204	254	195	172	332	277	286	12.0	
8/ 8/29	66	148	304	226	209	173	276	234	222	13.9	
8/ 9/29	67	145	306	300	292	230	442	612	636	8.5	
Average.....		131	235	232	222	200	352	352	349	10.3	

TABLE 4.—*Fasted Rabbits That Received No Insulin After Dextrose Feeding*

Date	Rabbit Number	Blood Sugar After First Dextrose: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose, per Cent	Comment
5/22/29	41	110	206	202	188	155	247	247	275	7.3	Slight diarrhea
5/26/29	42	145	400	434	472	408	728	964	1104	2.2	A slight drowsiness for last 3 hours
3/29/29	43	124	225	289	254	244	296	342	358	7.3	
4/ 2/29	44	113	264	275	239	198	213	292	366	5.5	
4/ 9/29	45	122	314	372	364	332	576	636	652	2.6	Weak and sleepy during last 3 hours; questionable slight diarrhea
8/30/29	82	145	298	364	346	300	460	576	552	8.2	
9/ 2/29	83	143	306	386	404	372	486	390	400	11.6	
9/ 2/29	84	132	300	350	340	304	368	412	346	10.1	
Average.....		129	289	334	327	289	422	482	507	7.0	

Liver Glycogen.—A comparison of tables 1, 2 and 3 shows that less glycogen was stored in the livers of animals receiving insulin than in the control rabbits. This is a confirmation of results obtained by many investigators. Whether "massive" doses or "small" doses of insulin were administered was immaterial so far as the glycogen content of the liver was concerned, probably because the "small" dose of 7 units per kilogram was sufficient to produce a maximum effect. In animals starved for forty-eight hours before the administration of dextrose and insulin, there again appeared to be some depression of the glycogen content of

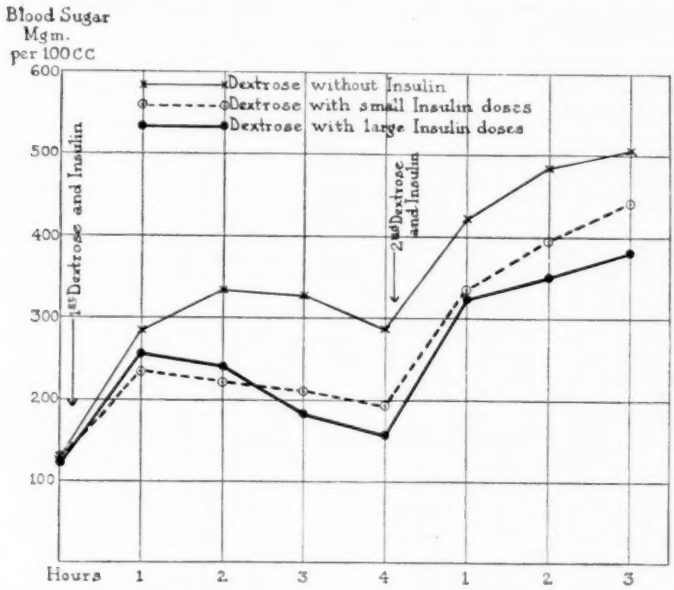

Chart 2.—Average blood sugar curves of previously fasting rabbits that received: (1) dextrose without insulin, (2) dextrose with "small" doses of insulin and (3) dextrose with "massive" doses of insulin.

the liver as contrasted with the animals receiving dextrose alone, as may be observed in tables 4, 5 and 6. The difference in behavior of insulinized and of control rabbits in this group is not as consistent or as striking as in the group of well-fed animals.

Pathologic Examination of Tissues.—The variations in weight of the adrenal glands and livers in relation to body weight were so great in the control animals that received dextrose but no insulin that no definite change could be noted in the rabbits receiving either "small" or "massive" doses of insulin. In the histologic studies of the adrenal

TABLE 5.—*Fasted Rabbits That Received Small Doses of Insulin (Seven Units Per Kilogram) After Each Feeding of Dextrose*

Date	Rabbit Number	Blood Sugar After First Dextrose and Insulin: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose and Insulin: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose and Insulin, per Cent	Comment
1/25/29	28	131	214	172	137	140	174	418	487	4.6	Somewhat weak the last 2 hours; liver slightly fibrotic
1/30/29	30	126	323	378	426	400	728	902	944	7.2	
2/ 5/29	32	128	197	177	168	175	307	340	376	8.2	Weak for last 2 hours
2/ 8/29	34	150	441	274	244	227	392	353	321	7.9	
2/19/29	36	131	158	210	276	258	577	667	703	2.5	Drowsy for last 3 hours
2/26/29	38	134	234	244	227	185	295	366	457	3.5	Rather drowsy
8/23/29	78	113	177	161	134	115	198	205	216	5.7	Stool rather soft; no diarrhea
8/27/29	80	145	247	243	199	172	213	215	358	5.5	
3/19/29	40	119	108	95	89	82	124	106	98	5.6	Stool rather soft; no diarrhea
8/30/29	81	141	255	258	198	182	346	...	...	....	Died 2 hours after second feeding of dextrose and insulin; stool soft
Average.....		132	235	221	210	193	335	397	440	5.6	

TABLE 6.—*Fasted Rabbits That Received Massive Doses of Insulin (Seventy-Five Units Per Kilogram) After Each Feeding of Dextrose*

Date	Rabbit Number	Blood Sugar After First Dextrose and Insulin: 0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	Blood Sugar After Second Dextrose and Insulin: 1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Liver Glycogen 7.5 Hours After First Dextrose and Insulin, per Cent	Comment
1/23/29	27	126	413*	444	359	308	523	618	698	6.2	
1/29/29	29	133	253	184	128	121	219	189	153	8.5	
2/ 1/29	31	122	309	235	163	131	372	374	458	5.7	
2/ 6/29	33	121	243	202	163	114	270	232	182	6.4	
2/15/29	35	120	166	210	162	169	366	394	461	4.6	Somewhat drowsy for last 3 hours
2/22/29	37	...	253	290	202	157	372	452	480	5.8	Somewhat drowsy for last 3 hours
8/22/29	76	134	264	211	131	106	181	288	385*	3.8	Weak in hind legs, apparently injured; liver slightly fibrotic
8/23/29	77	132	272	255	228	182	422	502	576	7.2	
8/27/29	79	134	177	134	106	108	238	168	154	5.8	
3/15/29	39	126	216	234	174	178	281	252	226	6.5	
Average.....		128	257*	240	182	157	324	347	377*	6.1	

* Approximate value.

glands, no changes were noted in the structure or in the lipoid and chromaffin content that could be attributed to the administration of insulin. No changes were observed in the structure or in the lipoid content of the livers of the insulinized animals.

TABLE 7.—*Well-Fed Rabbits That Received Small Doses of Crystalline Insulin After Each Dextrose Feeding*

		Blood Sugar After First Dextrose and Insulin					Blood Sugar After Second Dextrose and Insulin			
Date	Rabbit Number	0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Comment
4/24/29	47	146	196	185	155	155	...	...	...	Killed accidentally during second administration of dextrose
5/ 1/29	49	154	177	193	200	200	385*	502	528	Weak and dyspneic during last 3 hours
5/ 7/29	51	127	183	234	241	258	340	405	423	
5/17/29	54	151	188	147	137	...	228	283	220	
5/23/29	55	129	158	140	122	125	239	313	335	Rabbit died during the following night
Average.....		141	180	180	171	185	298*	376	377	

* Approximate value.

TABLE 8.—*Well-Fed Rabbits That Received Massive Doses of Crystalline Insulin After Each Dextrose Feeding*

		Blood Sugar After First Dextrose and Insulin					Blood Sugar After Second Dextrose and Insulin			
Date	Rabbit Number	0 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	4 Hr., Mg. per Cent	1 Hr., Mg. per Cent	2 Hr., Mg. per Cent	3 Hr., Mg. per Cent	Comment
4/23/29	46	131	180	146	123	103	...	...	...	Grew weak and died 1 hour after second feeding of dextrose and insulin
4/30/29	48	140	220	248	231	235	...	...	...	Died in convulsions 1 hour after second feeding of dextrose and insulin
5/ 3/29	50	120	197	217	212	168	356	500	...	Died in convulsions 2 hours after second feeding of dextrose and insulin
5/14/29	52	108	163	104	68	92	...	...	...	Rabbit killed accidentally during second administration of dextrose
5/15/29	53	148	248	210	113	131	293	385*	317	
Average.....		129	202	185	149	146	325	443*	317	

* Approximate value.

General "Toxic" Effects.—The amount of dextrose used to produce hyperglycemia was large and was given in concentrated solution. This caused the death of several control animals, all but one of which were in the well-fed group, which began the experiments with already loaded gastro-intestinal tracts. There was no greater mortality among the insulinized rabbits, even though some of them were given injections of

from 300 to 500 units within the space of four hours. Among the series of animals receiving "massive" doses of crystalline insulin, three of five died shortly after the injection of the second dose. Another rabbit in this series was killed accidentally. That death was not due to brucine poisoning, the only possible impurity that might result from the process of insulin crystallization, was shown by the fact that intravenous injections of several milligrams of this alkaloid had no effect on normal animals.

COMMENT

On the basis of the experimental work presented, it seems clear that even enormous quantities of insulin injected intravenously into normal rabbits, protected from hypoglycemia by dextrose feeding, manifest no general toxic effects in the course of seven and one-half hours. This observation is in harmony with the clinical observation of Byworth,[3] who used, with satisfactory results, 1,715 units of insulin in the course of thirty-six hours in the treatment of a patient with diabetic acidosis. No explanation can at present be offered for the high mortality of the rabbits treated with "massive" doses of crystalline insulin.

It can be definitely stated on the basis of the work presented in this paper that "massive" doses of insulin do not have a greater effect on the hyperglycemia resulting from the administration of dextrose than do "small" doses. This observation is in accord with the observations of Allan,[4] who in 1924 showed in depancreatized dogs that the dextrose equivalent per unit of insulin becomes progressively less as the dose of insulin is increased. Our experiments on well-fed rabbits even suggest that "massive" doses of insulin may, not only relatively but actually, have less effect on the hyperglycemia resulting from the administration of dextrose than do "small" doses of the hormone. The fact that no constant differences in the effect of "massive" and "small" doses of insulin were noted in the group of animals previously starved suggests that the amount of glycogen present in the tissues and the state of nutrition may play a significant rôle in the behavior of insulin administered after the ingestion of dextrose. It should be pointed out that the maximal rate of utilization of dextrose resulting from the injection of insulin is probably reached with doses less than 7 units per kilogram, which represents the "small" dose in our experiments.[5]

3. Byworth, H. A.: Brit. M. J. **1**:801, 1928.

4. Allan, F. N.: Am. J. Physiol. **67**:275, 1924.

5. Cori, C. F., and Cori, G. T.: J. Biol. Chem. **70**:557, 1926.

It has been observed by MacLeod,[6] Cori [7] and many others that insulin tends to suppress the deposition of glycogen in the liver after feedings of dextrose. Our work offers confirmation of these results. MacLeod has explained the low values for glycogen in the liver obtained following the injection of insulin by assuming that the small amount of blood sugar, which results from "a sugar vacuum" in the tissues, acts as a stimulus for glycolysis in the liver. This explanation fails to receive support from our experiments, since the liver of the insulinized rabbits contained smaller amounts of glycogen than did those of control animals, in spite of the presence of marked and persistent hyperglycemia. Cori and Cori [8] recently made observations similar to ours. From the preceding discussion it seems probable that the lower values for glycogen obtained in insulinized animals, in contrast to noninsulinized normal animals, result from an inhibition of the formation of glycogen in the liver or from the slightly lower concentrations of blood sugar.

In 1924, Riddle, Honeywell and Fisher [9] observed that a single large dose of insulin frequently caused marked swelling of the adrenal glands in pigeons protected from shock by adequate amounts of food. Poll [10] studied the adrenal glands of pigeons and rodents in insulin shock and found a marked decrease in the chromaffin and lipoid contents. Kahn [11] confirmed Poll's observations, and noted that section of the splanchnic nerves to one gland prevented the changes from developing in this organ. In our work the animals were always protected from shock, and no definite histologic changes were noted in the suprarenal glands. This would suggest that the changes reported by other investigators are dependent on the hypoglycemic reaction and not on the action of insulin per se.

CONCLUSIONS

1. A comparative study has been made of the effects of "small" and of "massive" doses of insulin in well-fed and previously starved normal adult rabbits protected from hypoglycemia by the ingestion of large amounts of dextrose.

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6. MacLeod, J. J. R.: Carbohydrate Metabolism and Insulin, New York, Longmans, Green & Company, 1926.

7. Cori, C. F., and Cori, G. T.: J. Biol. Chem. **70**:577, 1926.

8. Cori, C. F., and Cori, G. T.: J. Biol. Chem. **85**:275, 1929.

9. Riddle, O.; Honeywell, H. E., and Fisher, W. S.: Am. J. Physiol. **68**: 461, 1924.

10. Poll, H.: Med. Klin. **21**:1717, 1925.

11. Kahn, R. H.: Arch. f. d. ges. Physiol. **212**:54, 1926.

2. The marked variation in the blood sugar curves of rabbits receiving identical treatment has been emphasized.

3. "Massive" doses of insulin do not have a greater effect on the hyperglycemia resulting from administration of dextrose than do "small" doses. Evidence is presented which suggests that in well-fed rabbits "massive" doses of insulin have not only relatively but actually less effect on hyperglycemia than do "small" doses.

4. The storage of glycogen in the liver is suppressed by insulin in spite of the presence of persistent and marked hyperglycemia.

5. In contrast to observations made in the presence of hypoglycemia, no definite histologic changes are found in the adrenal glands of rabbits protected from insulin shock by dextrose. This suggests that the changes observed by other investigators were associated with hypoglycemia, and were not due to the action of insulin per se.

6. The injection of from 300 to 500 clinical units of insulin produces no general "toxic" effect in normal rabbits.

ACUTE TOXIC HEPATITIS (ACUTE YELLOW ATROPHY) DUE TO CINCHOPHEN

REPORT OF A CASE *

K. K. SHERWOOD, M.D.
REDMOND, WASH.
AND
H. H. SHERWOOD, M.D.
KIRKLAND, WASH.

Diagnosis in cases of painless jaundice is always an interesting problem. The two most common causes are, of course, catarrhal jaundice and carcinoma of the head of the pancreas. It so happened that last spring we had on our hospital service at one time three cases of jaundice, all of relatively rare etiology, namely, hepatic syphilis, hereditary hemolytic jaundice and cinchophen poisoning. In this paper we shall briefly review the literature and report the case of cinchophen poisoning.

Chemically, cinchophen is a ring compound containing nitrogen. This occurrence of nitrogen in its double benzene ring undoubtedly is the cause of the occasional development of toxic symptoms. Its therapeutic action is twofold:[1] first, as a mobilizer of uric acid, it remarkably hastens the excretion of uric acid through the kidneys; and second, it is a distinct antipyretic. This latter effect is marked, since a moderate dose will reduce the temperature of a normal dog an entire degree, whereas in a proportionate dose, sodium salicylate given to normal animals has no effect whatever.

Physiologically, it is excreted rather promptly in the urine, unchanged or slightly oxidized. It has been shown pharmacologically that benzene does not act as a hepatic poison. On the other hand, the inclusion of a nitrogen atom in the chemical ring makes possible the formation of nitrobenzene compounds from it by cleavage.[2] Trinitrophenol and trinitrotoluene are marked hepatic poisons. Crawford[3] studied the

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1. Furth, O., and Kuh, E.: Biochemical Studies on the Absorption of the Ethylester of Paramethylphenyl Cinchoninic Acid and Its Fate in Metabolism, J. Pharmacol. & Exper. Therap. **38**:1, 1930.

2. Brughsch and Horster: Toxicology of Atophan, Ztschr. f. d. ges. Med. **38**:386, 1923; quoted by Rabinowitz, M. A.: M. Clin. North America **11**:1025, 1928.

3. Crawford: TNT Poisoning (Case Report), Brit. M. J. **1**:450, 1918.

toxic symptoms of trinitrotoluene poisoning, and made the important discovery that in normal munition workers, trinitrotoluene was excreted in the urine. In three cases of acute yellow atrophy in munition workers, however, none was found in the urine, but it was found in the feces. The symptoms of trinitrotoluene poisoning as described by Crawford were essentially those of acute yellow atrophy. There was usually a prodromal stage of from one to three weeks before the onset of jaundice. Contrary to the course of acute yellow atrophy and of cinchophen poisoning, his three patients, although unconscious for many days (one of them for three weeks), recovered, and no residual bad effects could be found. He described the liver as markedly smaller than normal when the patients were in a comatose condition, and on full recovery, as again normal in size. Thus cinchophen poisoning is caused by the abnormal decomposition of the drug within the body into nitrocompounds, and by the failure of the kidneys to excrete these abnormal products of decomposition.

REVIEW OF THE LITERATURE

The drug was first introduced in 1908. It was not until 1913 that reports of adverse results in its use were first published. These dealt with the symptoms in the skin, which usually consist of a rash. The character of this rash is subject to great variation. In some patients it may assume the appearance of the rash in scarlet fever, in others it may be urticarial, and more rarely it is maculopapular. Varieties may or may not be accompanied by purpura-like spots. Several cases have been closely checked, and it has always been demonstrated that once the rash has appeared it will reappear with resumption of the use of the drug, even if a much smaller dose is given. Of the twelve cases with skin manifestations that have been reported, the only fatalities occurred in cases with marked symptoms in the liver.

Strangely enough, since 1913, little mention has been made of lesions in the skin. The first report of jaundice resulting from the use of this drug was that of Schraeder,[4] in 1922. He reported a series of seventeen cases, all of them mild and nonfatal. Gastro-intestinal symptoms, consisting mainly of nausea and heartburn, with painless jaundice accompanied by pruritis, are the features of the intoxication on which he laid stress. In passing, it is of interest to note that this series is over five times the size of any subsequently reported. Beginning in 1926, several reports are found each year. Langdon Brown's [5]

4. Schraeder, K.: Cases of Cinchophen Poisoning, Ugesk. f. laeger **84**:1141, 1922.

5. Brown, L.: Report on Atophan Poisoning, Brit. M. J. **2**:37, 1926.

report in July, 1926, seems to have drawn the attention of the scientific world to the possibility of fatal end-results following the administration of this drug. In Germany, several cases were the result of the intravenous administration of a preparation of cinchophen, or of giving an iodine preparation for the study of the gallbladder.[6]

We have been able to gather from the available literature [7] fifty-four cases—fifteen fatal, thirty-nine attended with recovery—clinically complete save in one instance. There seems to be no especial sex or age predilection, if one considers the fact that elderly people present more symptoms for which this drug will be used. All except one of the patients with gastro-intestinal symptoms had jaundice. In a few cases, there was a considerable lapse of time between the cessation of the administration of the drug and the inception of the symptoms. Nausea, heartburn and, in most cases, tenderness in the upper right quadrant of the abdomen preceded the jaundice by from twenty-four hours to five days. There does not seem to be any relation between the daily dose or the length of time the drug was taken and the occurrence of toxic symptoms. This, however, could only be determined by study of a large series in which varying doses would be given over a long period of time. The largest amount taken before toxic symptoms developed was 7,000 grains (455 Gm.), the least, 15 grains (0.97 Gm.); the most taken in one day was 70 grains (4.5 Gm.), the least, probably 15 grains, although symptoms developed in several patients when an average dose of less than 15 grains per day was given over a period of weeks.

CLINICAL PICTURE

The clinical picture of cinchophen poisoning is that of acute yellow atrophy of the liver. The first symptoms to be complained of are usually gastric irritability, intolerance and more rarely heartburn. Nausea is almost universally present. It is important to know that these symptoms may arise as late as ten days after the cessation of the administration of the drug. In from one day to a little over a week after these symptoms, the onset of jaundice, biliuria, pruritis and sometimes clay-colored stools occur. Pain is usually absent, and when it does occur, it is not extreme. The amount of the drug taken seems to have little bearing on the onset or course of the disease. The jaundice continues to deepen, and usually if it is looked for, a decrease in the

6. Hitzenberger, K.: Atophan Poisoning (Case Report), Wien. klin. Wchnschr. **40**:205, 1927; Singer, S.: Ibid., p. 238. Schwartz, G.: Ibid., p. 238. Haudek, M.: Ibid., p. 239. Klinkert, D.: Atophan Poisoning (Case Report), Therap. d. Gegenw. **69**:140, 1928.

7. The articles by Dassen, Miller and Rake (accompanying table) and that by Singer (footnote 6) were not available in the original. These cases were included in the table because they were quoted elsewhere in sufficient detail.

size of the liver can be demonstrated. In the more chronic cases, ascites and splenomegalia may develop. Thus, jaundice usually occurs in the first week of the illness; if by the end of a second week of jaundice, there is not a distinct improvement in the patient's condition, the third week will usually show a marked increase in the symptoms: jaundice and the toxemia increase, the liver decreases in size, and edema appears in the extremities. Usually by the fourth week the edema begins to become generalized. This is of especially grave significance.

Table on the Cases of Cinchophen Poisoning Reported in the Gastro-Intestinal Literature

Author	Number of Cases	Involvement		Died	Recovered
		Skin	Gastro-intestinal		
Anderson, S. O., and Teter, O. P.: J. A. M. A. **93**: 2 (July 13) 1929	1	..	1	1	..
Brown (footnote 5)	2	..	2	2	..
Dassen, R.: Semana méd. **36**: 368, 1929; abstr. J. A. M. A. **92**: 1563 (May 4) 1929	2	..	2	..	2
Drought-Worster, C.: Brit. M. J. **1**: 148, 1923	1	1	1	..	1
Evans, G.: Brit. M. J. **2**: 93, 1926	3	..	3	..	3
Frenzel, W. C.: Wisconsin M. J. **28**: 264, 1927	1	..	1	..	1
Glover, L. S.: Brit. M. J. **2**: 136, 1926	1	..	1	..	1
Haudek (footnote 6, fourth reference)	1	..	1	..	1
Herrick, W. W.: J. A. M. A. **61**: 1376 (Nov. 11) 1913	1	1	1	..	1
Hitzenberger (footnote 6, first reference)	2	..	2	2	..
Lowenthal, L. J. A., Mackay, W. A., and Lowe, F. C.: Brit. M. J. **1**: 592, 1928	2	..	2	2	..
McVicar, C. S., and Wier, J. F.: M. Clin. North America **12**: 1526, 1929	1	1	1	1	..
Miller, M.: Gesellsch. f. inn. Med. u. Kinderh., Feb. 13, 1913; abstr. Therap. Monatschr. **27**: 468, 1913	1	1	..	..	1
Petty, M. J.: Brit. M. J. **2**: 442, 1928	1	..	1	..	1
Phillips, J.: J. A. M. A. **61**: 1040 (Sept. 27) 1913	5	5	..	..	5
Rabinowitz, M. A.: M. Clin. North America **11**: 1025, 1928	2	..	2	..	2
Rake, G. W.: Guy's Hosp. Rep. **77**: 229, 1927	1	..	1	1	..
Reichle, H. S.: Arch. Int. Med. **44**: 2 (Aug.) 1929	2	2	2	2	..
Schraeder (footnote 4)	17	..	17	..	17
Schwartz (footnote 6, third reference)	1	..	1	..	1
Singer (footnote 6, second reference)	1	..	1	1	..
Sutton, D. C.: J. A. M. A. **91**: 310 (Aug. 4) 1928	1	1	1	1	..
Wells, C. L. L.: Brit. M. J. **2**: 759, 1926	1	..	1	1	..
Wilcox, W. H.: Brit. M. J. **2**: 273, 1926	3	..	3	1	2
	54	12	48	15	39

As far as can be ascertained from incomplete case records, no patient in whom this occurred has recovered. Death frequently occurs without the development of edema, however. The patient becomes delirious, then comatose, and dies, presenting a striking picture of hepatic insufficiency. Fever is usually absent, and when present may be accounted for either by a secondary factor, e. g., bronchopneumonia, or by the process for which the drug was given.

Pathologically, the most striking picture is seen in the liver,[8] which is very small and soft. In the sections, yellow areas the size of a

8. Reichle, H. S.: Toxic Cirrhosis of the Liver, Arch. Int. Med. **44**:2 (Aug.) 1929.

pinhead are surrounded by soft, red, beefy tissue. Microscopically, one scarcely recognizes the tissue as hepatic. The red areas are found to consist of necrotic débris, small round cells, young connective tissue and fat, free and intercellular. The yellow areas, which contain the only recognizable hepatic cells, are small, and the vast majority of the cells contain a droplet of fat which displaces the greater amount of its area. What remaining cytoplasm there is, is granular. The fatty degeneration is not limited to the liver, however. It is also conspicuous in the heart muscle and in the kidney. Splenomegalia is only seen in the more chronic cases. Edema of the extremities and serous cavities may be great. Primary hepatolysis, therefore, can be accompanied by secondary fatty degeneration of the heart and kidneys. The only exception is the case of Petty,[9] which at operation showed a pancreatic necrosis. This patient recovered, so no pathologic examination of the liver was possible.

Is there anything that can be done for this condition? Certainly its recognition earlier and the discontinuance of the drug are of primary importance. Graham,[10] in a paper read before the Royal Society in London, emphasized the fact that there is a correct and an incorrect way to administer the drug. He recommended as the correct way, the giving of moderate doses, accompanied by alkalis, for periods of four days, with at least a three day rest between series. It is perhaps significant that only one case of poisoning is reported wherein this regimen was followed. The first gastro-intestinal symptoms in a patient taking a cinchophen preparation should be the signal for its permanent discontinuance—permanent, because it has been repeatedly shown that once symptoms are started, the readministration of even a single dose of the drug may bring on jaundice or symptoms in the skin, if not a fatal outcome. This is preventive therapy. Once jaundice is established, physicians must place their main reliance on dextrose. Mann[11] has shown that the cause of death in experimental removal of the liver is due, at least primarily, to the inability of the organism to keep the blood sugar level at its optimum. It is one of the most important functions of the liver to serve as a reservoir for the storage of dextrose so that it can be taken as needed to keep the blood sugar at a nearly constant level. Thus, by frequently giving our patients carbohydrates, we are minimizing the amount of work the liver must perform. Elimination should be aided, and the intake of fluids augmented to the amount

9. Petty, M. J.: Atophan Poisoning (Case Report), Brit. M. J. **2**:442, 1928.

10. Graham, G.: Presidential Address, Section of Therapeutics and Pharmacology, Tr. Roy. Soc. Med., October, 1926, quoted by Lowenthal (see table).

11. Mann, F. C.: Effects of Complete and Partial Removal of the Liver, Medicine **6**:419, 1927.

that will be excreted. Rest in bed is, we feel, essential for conserving to the utmost both heart and kidneys, which frequently show a fatty degeneration. Treatment of edema or ascites should be conservative. Sutton's [12] experience with giving a mercury diuretic intravenously illustrates the dangers of treating accumulated fluid in this disease in a routine manner. He administered such a compound with the result that the output of urine, which had been about 1,400 cc. per day, promptly dropped to 300 cc. for the next three and a half days. Usually in the absence of a fatal ending, results are good. Only one case has been reported in which the patient was left with clinical splenomegalia, and even that was of a mild degree (the spleen was just palpable).

REPORT OF A CASE

History.—W. M., a man, aged 27, was first seen on Jan. 4, 1930. His personal and family history were unimportant. The patient complained of a gonorrheal infection and he was given the usual treatment, with seemingly satisfactory results, until January 21, when severe cystitis developed, with almost a constant straining to void. This yielded to irrigations of the bladder, so that by February 1 he was in a satisfactory condition, and it seemed that he was over his troubles. On February 13, however, he began to complain of pain in his ankles and feet, which soon shifted and centered in the left hand and wrist; the pain was so severe that the patient could obtain no rest. With the onset of pain, the temperature rose to 100 F. and then to 103 F. After a few days on salicylates and iodides he became comfortable, and the temperature dropped almost to normal. On February 20, he had a chill followed by high temperature, and the recurrence of great pain in the left hand and wrist. The pain was so severe that we gave him a hypodermic of morphine sulphate, ¼ grain (0.016 Gm.), thinking that it would give him relief, but it did not; so the following night hyoscine morphine cactin anesthesia was tried. This resulted in a wild delirium. He was put on cinchophen tablets (Schering and Gatz), taking 75 grains (4.87 Gm.) in all. He was free from pain, and his temperature was normal. He remained comfortable for two days, then on the night of February 26 (five days after the administration of cinchophen had been commenced), he had a severe chill and great pain in the region of the right hip. At this time he was also somewhat jaundiced. He was then persuaded to go to the hospital, where he was admitted on February 29, complaining of great pain and marked jaundice.

Course.—On March 1, the patient was very uncomfortable. The stools were clay-colored. Examination of the blood revealed: hemoglobin, 70 per cent; red cells, 3,860,000; white cells, 14,150 with 79 per cent polymorphonuclears. The Wassermann reaction was negative, and urinalysis showed bile. Both the van den Bergh immediate and delayed reactions were positive. The icterus index was 70. On March 3, the van den Bergh biphasic reaction was positive and the icterus index was 70. The patient was still very uncomfortable on the following day. The van den Bergh direct quantitative reaction was + 7.5 mg. of serum. The stools were slightly positive from the bile. On March 7, the patient was more comfortable, his condition was less toxic, and the jaundice was beginning to clear.

12. Sutton, D. C.: Acute Yellow Atrophy of Liver Following Taking of Cinchophen (Case Report), J. A. M. A. **91**:310 (Aug. 4) 1928.

The next day the van den Bergh direct reaction was + 3.4 mg. of serum. The temperature ranged from normal to 100 F. Continued improvement was observed on March 10, and on the twelfth, intravenous salicylates were started, which gave marked relief. From the fourteenth to the twenty-sixth, there was gradual improvement on intravenous salicylates and a diet high in carbohydrates.

The patient was discharged on March 28, able to walk on crutches. The jaundice had disappeared.

On April 20, no symptoms had recurred, and he was pronounced clinically well.

CONCLUSIONS

1. The administration of cinchophen is occasionally accompanied by toxic symptoms.

2. These toxic symptoms are of two types, affecting either the skin or the liver.

3. Twelve cases in which there were skin manifestations have been collected from the literature; unless complicated by symptoms in the liver, none of these was fatal. Once cutaneous symptoms appear, the readministration of the drug, even in smaller doses, will cause their recurrence.

4. Forty-eight cases of acute hepatic necrosis were collected from the literature, and one case was added. Fifteen fatalities were reported. Poisoning is apparently due to an abnormal decomposition of cinchophen in the body and its retention, thus corresponding to that occurring in munition workers (due to trinitrotoluene poisoning).

5. Neither the length of time the drug was administered nor any save excessive doses (70 grains) appear to predispose to the onset of toxic symptoms.

6. Pathologically, in the fifteen cases in which death occurred, acute hepatic necrosis (acute yellow atrophy) was found with acute fatty degeneration of the heart and kidneys.

7. Therapy consists of complete rest in bed and the forced feeding of fluids and carbohydrates.

8. Only one patient who recovered retained clinical signs of portal stasis (splenomegalia). Readministration of the drug, even in much smaller doses, will usually produce jaundice immediately.

ESSENTIAL HYPERTENSION

THE DIASTOLIC BLOOD PRESSURE: ITS VARIABILITY *

DAVID AYMAN, M.D.

BOSTON

There have been in the past many unemphasized observations of diastolic variability both in patients with arterial hypertension and in those with normal blood pressure. In 1911, Schrumpf and Zabel [1] found that in their arteriosclerotic patients, in which group they included those with arterial hypertension, the diastolic blood pressure fluctuated almost parallel with the systolic. They noted diastolic variations up to 35 cm. (water sphygmomanometer). Tixier [2] mentioned that the diastolic tension may undergo important variations. More recently, Fahrenkamp,[3] referring to a case of "malignant nephrosclerosis," stated that the diastolic blood pressure curve showed fundamentally the same fluctuations as the systolic curve. Kylin,[4] in a study of the lability in essential hypertension, does not discuss diastolic lability, but in a charted example of the blood pressure of a patient with essential hypertension he shows a variation of 55 mm. of mercury in the diastolic blood pressure during a week's observation. During a twenty-four hour period of study, Mueller and Brown [5] found a diastolic variation of 40 mm. of mercury in a patient with normal blood pressure and a diastolic variation of 70 mm. in a patient with essential hypertension. In a patient with "severe benign hypertension," Brown [6] found a diastolic variation of 48 mm. within a five hour period. Frost [7] and Amiral [8]

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* From the Medical Clinic of the Boston Dispensary, service of Dr. Joseph H. Pratt, and the Division of Research.

1. Schrumpf, P., and Zabel: Die diagnostische Bedeutung der psychogen Labilität des Blutdruckes, München. med. Wchnschr. **58**:1952 (Sept. 12) 1911.

2. Tixier, L.: Les variations normales et abnormales de la tension artérielle humérale, Arch. d. mal. du coeur **12**:337 (Aug.) 1919.

3. Fahrenkamp, K.: Die psycho-physischen Wechselwirkungen bei den Hypertonierkrankungen, Stuttgart, Hippokrates, 1926, p. 42.

4. Kylin, E.: Die Hypertoniekrankheiten, Berlin, Julius Springer, 1926, p. 70.

5. Mueller, S. C., and Brown, G. E.: Hourly Rhythms in Blood Pressure in Persons with Normal and Elevated Pressures, Ann. Int. Med. **3**:1190 (June) 1930.

6. Brown, G. E.: Daily and Monthly Rhythm in the Blood Pressure of a Man with Hypertension: A Three-Year Study, Ann. Int. Med. **3**:1177 (June) 1930.

7. Frost, H. M.: The Response of the Cardio-Vascular System to Respiratory Exertion, Boston M. & S. J. **191**:853 (Nov. 6) 1924.

8. Amiral, H. H.: The Reaction of the Diastolic Pressure to Respiratory Strain, Boston M. & S. J. **191**:863 (Nov. 6) 1924.

produced, both in patients with normal blood pressure and in those with hypertension, diastolic variations up to 32 mm. of mercury by means of respiratory exertion. O'Hare and Walker,[9] while observing the effect of salt in essential hypertension, found in patients in the wards diastolic variations up to 65 mm. O'Hare stated [10] that excitement might raise the diastolic blood pressure as much as 24 mm. While discussing "emotional hypertension," Stieglitz [11] reported an average rise of 29 mm. in the diastolic blood pressure of eight patients under emotional strain. Mosenthal and Short [12] and Sigler [13] separately studied the effect of rest on the blood pressure in essential hypertension by taking the blood pressure of reclining subjects at frequent intervals during total periods of from three to ninety minutes. Mosenthal and Short found in forty of these patients an average drop in diastolic blood pressure of 10 mm. of mercury, with ranges of 0 to 30 mm. Sigler did not publish the details of his studies, but he charted a diastolic variation of 50 mm. of mercury, occurring in a patient while reclining for eight minutes.

In almost all of the foregoing studies, the problems investigated have not been those of diastolic variability, so that the diastolic variations are hardly more than mentioned. All the observations cited, among many others unquoted, show that there is a significant fluctuation of the diastolic blood pressure in individual cases. These studies do not indicate, however, the great extent to which the diastolic blood pressure may fluctuate in a large number of patients with essential hypertension who have been observed over a moderately long period of time. Such is the nature of the present study.

METHOD OF PRESENT STUDY

During the past two years, I have seen a large group of patients with essential hypertension at frequent intervals in the hypertension clinic of the Boston Dispensary. Of this group, seventy-six patients were chosen for the purpose of the present study. The basis of selection was merely that of regular attendance at

9. O'Hare, J. P., and Walker, W. G.: Observations on Salt in Vascular Hypertension, Arch. Int. Med. **32**:283 (Aug.) 1923.

10. O'Hare, J. P.: Vascular Reactions in Vascular Hypertension, Am. J. M. Sc. **159**:373 (March) 1920.

11. Stieglitz, E. J.: Emotional Hypertension, Am. J. M. Sc. **179**:775 (June) 1930.

12. Mosenthal, H. O., and Short, J. J.: The Spontaneous Variability of Blood Pressure and the Effects of Diet upon High Blood Pressure, Am. J. M. Sc. **165**: 531 (April) 1923.

13. Sigler, L. H.: Spontaneous Nonrhythmic Variations in the Blood Pressure Levels and in the "Silent Gap," Am. J. M. Sc. **177**:494 (April) 1929.

the clinic. The diagnosis of essential hypertension was made in the individual case only after at least two months' observation and after obtaining at least five abnormally high blood pressure readings, together with normal renal function tests.[14] A few of the patients observed had moderately impaired renal function. These have been purposely included in the study and will be referred to later. No medication was given during the period of observation utilized for the present investigation.

I made all the blood pressure observations by using a mercury sphygmomanometer, the auscultatory method, and the beginning of the fourth sound as an index of the diastolic blood pressure. The blood pressure readings were taken in a quiet room while the patient was seated, with his arm resting on an adjacent table. The readings were taken at each visit when the patient first sat down and then at intervals of from five to ten minutes over periods varying between five and forty-five minutes. In this manner, patients were observed over periods of from two months to two years, although the period of known hypertension ranged in the individual cases from a minimum of seven months to a maximum of fifteen years. In determining the total individual diastolic variation during the period of study, the highest diastolic reading was utilized only if it antedated or was taken soon after the lowest diastolic reading. In this way, one obviates the criticism that as the disease advances during the course of a year or two, the diastolic blood pressure may rise naturally and an apparent fluctuation appear.

OBSERVATIONS

For the purpose of analysis, the seventy-six patients were divided into three groups on the basis of the general height of their systolic blood pressure, group 1 having readings usually above 200 mm. of mercury, group 2 usually between 160 and 200 mm. of mercury, while in group 3 the systolic blood pressure readings were mostly between 160 mm. and the upper normal limits.[15] The diastolic blood pressures of group 1 were in general higher than those of group 2, which, in turn, were higher than those of group 3.

1. *Total Individual Variations Without Rest Periods.*—The total diastolic variations in these three groups ranged from a minimum of 5 mm. to a maximum of 66 mm. of mercury, with an average variation for the seventy-six patients of 30 mm. The average variation of group 1 was 37.5 mm.; of group 2, 32.4 mm., and of group 3, 22.5 mm. (table 1). In other words, the higher the systolic blood pressure, the greater was the total diastolic fluctuation in these patients. The total variation of the diastolic blood pressure in each of the seventy-six patients is shown in chart 1. So far these figures refer only to the total diastolic variation found in the initial blood pressure readings of all visits, excluding the drops due to rest periods.

14. Ayman, D.: Normal Blood Pressure in Essential Hypertension, J. A. M. A. **94**:1214 (April 19) 1930.

15. Hunter, A. W.: Blood Pressure: What Affects It? An address before Association of Life Insurance Presidents, Dec. 6, 1923.

2. *Effect of Rest Periods on the Total Variation.*—By including in the total variations the drops in diastolic blood pressure that occurred while the patient sat in a quiet room, it was found that the diastolic variations in the three groups ranged from a minimum of 8 mm. to a maximum of 70 mm., with an average of 37 mm. (chart 1 and table

TABLE 1.—*The Effect of Rest and of the Height of the Systolic Blood Pressure on the Total Diastolic Variation*

	Group 1: 24 Patients With Systolic Usually 200 Mm. or More	Group 2: 22 Patients With Systolic Usually Between 160-200 Mm.	Group 3: 30 Patients With Systolic of 160 Mm. or Less
Total diastolic variation of initial blood pressure readings during total observation	37.5 mm.	32.4 mm.	22.5 mm.
Total diastolic variation of all diastolic readings during total observation (including rest periods)	43.5 mm.	37.5 mm.	30 mm.

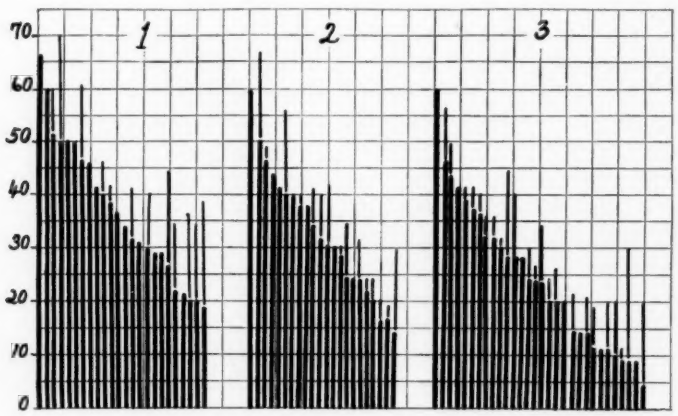

Chart 1.—The total variation of the diastolic blood pressure in each of seventy-six patients. Each vertical black band represents the total variation occurring without rest periods. The narrower vertical bands indicate the additional diastolic variation due to rest periods. Groups 1, 2 and 3 correspond to those described in the text.

1). The average variation of group 1 now became 43.5 mm.; of group 2, 37.5 mm., and of group 3, 30 mm. Rest periods, therefore, increase the total diastolic variations.

3. *Relation of the Number of Visits to the Amount of Total Variation.*—The extent of the total fluctuation in the individual case bears a distinct relation to the total number of visits made by the patient and utilized for the study: the greater the number of visits, the greater

the fluctuation within limits. This is shown in chart 2 which illustrates only the patients in group 1. The same relationship was found to hold true in groups 2 and 3. Practically all patients observed ten or more times had total diastolic variations of 30 mm. of mercury or more.

4. *The Percentage of Variation of the Diastolic as Compared with the Percentage of Variation of the Systolic Blood Pressure.*—By taking the highest diastolic blood pressure reading in the individual case, e. g., 150 mm. of mercury, and also the corresponding lowest diastolic reading, e. g., 100 mm., it is possible to express the difference of fluctuation as a percentage fluctuation of 150 mm. By using the same method with

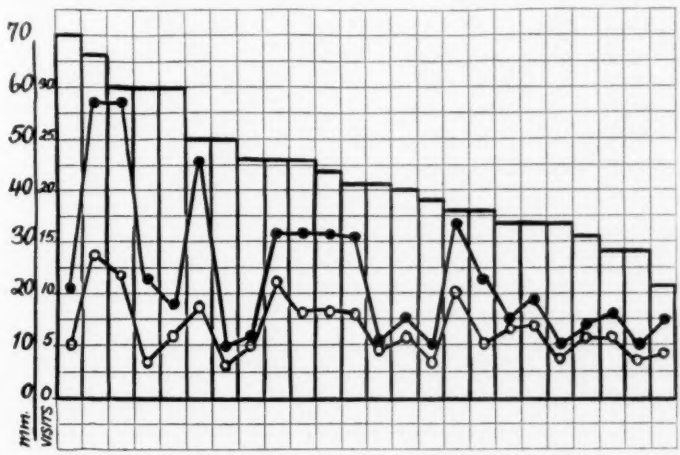

Chart 2.—The relation of the number of visits to the degree of total diastolic fluctuation. The chart is a rearrangement of the cases in chart 1 (*1*) to show the entire diastolic fluctuation in each case. The total number of visits (solid black dots) and the number of visits with rest periods (circles) are plotted in each case.

the corresponding systolic blood pressures, it is then possible to compare the percentage of fluctuations of the systolic and diastolic blood pressures. It is thus seen that a patient whose blood pressure has varied, for example, between 240 systolic and 150 diastolic and 160 systolic and 100 diastolic, has had a percentage fluctuation of 33.3 per cent in both the systolic and the diastolic blood pressures.

In this manner the percentage fluctuations of the systolic and diastolic blood pressures of the seventy-six patients were determined and compared in each patient. It was found that in group 1 the average percentage fluctuation of the diastolic pressure was 32 per cent and of the systolic, 32 per cent; in group 2, the diastolic was 32 per cent and

the systolic was 36 per cent, and in group 3, the diastolic was 26 per cent and the systolic was 27 per cent. The average percentage fluctuation for the three groups was diastolic, 30 per cent and systolic, 31.6 per cent. For practical purposes it may be said that the percentage fluctuation of the diastolic blood pressure was as great as that of the systolic blood pressure.

5. *Maximum Diastolic Variations at Any One Visit.*—In order to determine the maximum diastolic variation at any one visit in each patient, it was necessary to utilize only the blood pressure readings taken during visits with rest periods. It was found that the greatest diastolic fluctuation at any one visit was 65 mm. and the smallest variation was 4 mm. The average of group 1 was 17.9 mm.; of group 2, 13.5 mm., and of group 3, 14.5 mm. The modal distribution of these fluctuations showed that in group 1, 82 per cent of the patients had maximum fluctuations between 15 and 25 mm.; in group 2, 81 per cent had maximum variations between 5 and 20 mm., and in group 3, 84 per cent had their maximum between 5 and 20 mm.

6. *The Relation of the Maximum Diastolic Variation at Any One Visit to the Number of Visits with Rest Periods.*—Of the patients who made more than five visits with rest periods, 65 per cent (twenty-three) had maximum diastolic variations of more than 15 mm. at one of these visits. On the other hand, of the patients who made five or less visits with rest periods, 65 per cent (twenty-seven) had maximum diastolic variations of less than 15 mm. of mercury at one of these visits. This indicates that the greater the number of visits with rest periods, the greater the maximum diastolic variation during some one of these visits.

7. *The Relation Between the Maximum Diastolic Variation at Any One Visit and the Time Required for the Variation to Occur.*—The maximum diastolic variation at any one visit occurred in 80 per cent of the patients only after ten minutes or more of rest and in 50 per cent of the patients only after twenty minutes or more of rest. However, the amount of maximum variation at any one visit seemed to bear no relation to the length of the rest period. Of the patients who had rest periods of less than ten minutes, 60 per cent had variations of more than 15 mm.; of the patients who had rest periods of from ten to twenty minutes in length, 50 per cent had variations of more than 15 mm., and of the patients who had rest periods of more than twenty minutes, 46 per cent had variations of more than 15 mm.

COMMENT

In seventy-six unselected, untreated patients with essential hypertension, the diastolic blood pressure was found to fluctuate widely

TABLE 2.—*Analysis of the Seventy-Six Cases of Essential Hypertension in which the Diastolic Blood Pressure Fluctuated* *

Case	Age	Known Duration of Hypertension, Yr.	Diastolic Range During Total Observation	Heart Enlargement by Orthodiagraph	Electrocardiographic Changes, Degree	Changes in Retinal Blood Vessels	Renal Function Impairment
			Group 1				
1	51	5.0	150- 84	—	—	+++	—
2	44	3.0	150- 90	—	+30	+++	—
(a) 3	42	2.0	154- 94	±	+16	+++	—
4	59	2.0	160- 90	+	+33	++	—
(b) 5	64	12.0	118- 68	±	Fibrillation	++	—
6	40	5.0	140- 90	+	+24	+++	—
7	64	4.0	140- 80	+	..	+++	—
8	64	1.0	134- 88	..	..	+++	—
(a) 9	67	1.3	140- 98	—	..	Cataracts	—
10	70	7.0	120- 74	++	+ 6	+++	—
11	50	8.0	130- 88	+	0	+++	—
12	51	11.0	136-100	++	— 3	+++	+
13	61	1.2	120- 86	—	—	++	—
14	68	5.0	140- 94	+	+24	+++	—
(c) 15	50	2.2	140-108	++	+30	+++	—
(b) 16	53	0.6	140-100	+++	..	+++	—
17	59	2.0	130-102	..	..	+++	—
18	46	8.0	138-110	+	—	+++	—
19	61	15.0	124- 80	..	+40	+++	—
20	64	1.5	128- 94	+	..	+++	—
21	69	8.0	122-100	+++	..	+++	+
22	44	6.0	144-108	+	+35	++	—
23	63	4.1	130- 96	++	+20	+++	—
(b) 24	62	3.8	140-102	++	..	..	+
			Group 2				
1	53	1.0	140- 80	—	..	+	—
2	52	1.5	140- 74	+	+38	—	—
3	42	1.5	150- 92	+	+38	++	—
4	50	7.0	124- 80	—	— 4	++	—
5	54	6.2	136- 94	+	..	—	—
6	63	1.3	128- 72	..	..	++	—
(a) 7	56	2.0	130- 90	—	+25	++	—
8	46	1.5	130- 90	+	+ 3	++	—
9	62	5.0	110- 72	+	— 2	++	—
10	65	4.0	102- 60	—	..	++	—
11	70	5.6	100- 60	—	..	+++	—
12	75	5.0	102- 60	+	+11	+++	—
13	56	6.0	120- 90	—	..	+	—
14	51	6.5	100- 70	..	..	—	—
15	70	1.6	118- 70	..	..	—	—
16	68	1.2	108- 84	++	..	+++	—
17	77	6.0	118- 80	++	+16	+++	—
18	61	1.5	100- 76	+	..	+	—
(a) 19	69	4.2	100- 76	+	..	+++	—
20	77	7.0	110- 90	..	0	Cataracts	—
21	60	9.0	120-102	—	..	++	—
22	62	7.5	120- 90	—	—14	++	—
			Group 3				
1	45	11.0	140- 80	+	—16	++	—
2	53	10.0	124- 70	—	—	—	—
3	54	4.0	130- 82	—	—	++	—
4	52	2.0	110- 68	—	+ 8	++	—
5	46	1.5	120- 78	..	..	++	—
6	54	1.3	110- 68	—	..	+	—
7	75	2.1	108- 68	+	..	+++	—
8	56	1.2	100- 64	—	—	+++	—
9	45	7.0	112- 76	—	..	++	—

TABLE 2.—*Analysis of the Seventy-Six Cases of Essential Hypertension in which the Diastolic Blood Pressure Fluctuated*—Continued*

Case	Age	Known Duration of Hypertension, Yr.	Diastolic Range During Total Observation	Heart Enlargement by Orthodiagraph	Electrocardiographic Changes, Degree	Changes in Retinal Blood Vessels	Renal Function Impairment
			Group 3—Continued				
10	25	1.0	100- 68	..	..	..	—
11	74	4.0	104- 60	+	—10	+++	—
12	51	5.0	110- 70	++	..	+++	—
13	49	2.0	120- 92	—	..	++	—
14	70	1.8	104- 74	—	..	++	—
15	19	1.0	98- 72	..	..	..	—
16	60	3.5	102- 68	—	—	++	—
17	48	1.3	104- 80	—	0	++	—
18	52	7.0	110- 84	.	— 6	—	—
19	38	2.2	128-108	—	..	++	—
20	58	6.2	90- 68	—	..	+	—
21	50	0.5	92- 74	..	--	+++	—
22	44	1.0	110- 90	—	..	++	—
23	30	0.2	110- 92	..	..	..	—
24	19	0.8	88- 76	..	..	—	—
25	46	1.1	120-100	—	..	+	—
26	52	7.0	108- 90	—	..	+++	—
27	34	0.2	102- 90	..	..	..	..
28	43	1.0	120- 90	—	..	—	—
29	56	1.2	104- 96	..	..	..	—
30	54	1.0	104- 84	—	..	++	—

* In the table +++ indicates marked cardiac enlargement, or retinal arteriolar sclerosis; ++, moderate cardiac enlargement, or retinal sclerosis; +, slight cardiac enlargement, or retinal sclerosis; under electrocardiographic changes are given the degree of left axis deviation according to the standards of Einthoven.

(a) Have had a cerebral hemorrhage.
(b) Died of cerebral hemorrhage.
(c) Died of cardiac insufficiency.

during periods of observation extending in the individual cases up to one and a half years. It might possibly be contended that the author was dealing with a mild type of essential hypertension. Table 2 shows, however, that many of the patients had already been severely injured by the disease.

In this table, the patients have been arranged in the individual order of the three groups in chart 1. It is seen that in group 1 all the patients had marked retinal arteriolar sclerosis;[14] practically all of them had cardiac enlargement determined by the orthodiagraph, and many of them had electrocardiographic abnormalities. Twenty-five per cent, or six patients, have died or have had cerebral hemorrhages. Seventeen per cent, or four patients, show moderate renal damage, as indicated by poor ability in dilution and concentration, together with albumin and casts. Yet the patients in group 1, despite their generally higher levels of blood pressure than those in groups 2 and 3, and despite their advanced changes, including renal, had, both absolutely and by percentage, as great a diastolic variation as those in groups 2 and 3. In group 2 there is a smaller degree of vascular organic changes, yet sufficient to cause cerebral hemorrhage in two patients. There was as great a diastolic fluctuation in group 2 as in groups 1 and 3.

It therefore cannot be denied that patients with even advanced essential hypertension may, and usually do, have marked fluctuations in their diastolic blood pressure from visit to visit. The degree of fluctuation depends to a certain extent on the number of observations made on the patient: the more frequent the observations, the greater the fluctuation recorded. The lowering effect on the diastolic blood pressure of sitting in a quiet room also seems definite and, conversely, the elevating effect of excitement.

It also is seen that in these observations no patient had a fixed, nonfluctuating hypertension. The systolic and diastolic blood pressures fluctuated in all cases, including the few with definite renal involvement. This suggests that, perhaps outside of some cases with extremely advanced so-called malignant hypertension, practically all patients with essential hypertension have fluctuating blood pressures, and as much, by proportion, in the diastolic as in the systolic blood pressure.

CONCLUSIONS

The diastolic blood pressure in essential hypertension, when observed frequently and over a sufficiently long period, fluctuates over a wide range. The percentage of fluctuation of the diastolic is as great as that of the systolic blood pressure.

484 Commonwealth Avenue.

CINCHOPHEN OXIDATION TEST OF THE FUNCTION OF THE HEPATIC CELLS*

S. S. LICHTMAN, M.D.
NEW YORK

It is the present consensus [1] that none of the current tests of the function of the liver determine accurately the functional capacity of the organ as a whole. The value of the more useful tests, such as the van den Bergh reaction, the icterus index and the Rosenthal test, is vitiated by the presence of obstructive jaundice even of slight degree, because these tests depend on the measurement of the capacity of the liver to excrete bilirubin or bromsulphalein. Yet in the presence of marked obstructive jaundice, the nonexcretory functions of the liver may remain relatively undisturbed. The more common of the so-called metabolic tests of the function of the liver, such as the determination of the tolerance for galactose and levulose, unfortunately do not give uniform results, and at best permit only of qualitative statements.

Therefore, a test that would determine more accurately the degree of functional impairment of the liver in disease must be one that is not materially influenced by disturbances in the excretory functions of the liver. With this criterion in mind, I have studied the metabolism of phenylcinchoninic acid, a substance known to act as a specific stimulant to the hepatic cells and to possess the properties of a "choleretic." [2]

After the ingestion of cinchophen, a product of oxidation identified as 2-(ortho-hydroxy)-phenyl-quinoline-4-carboxylic acid appears in the urine.[3] I have devised a simple colorimetric method for the estima-

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* From the Laboratories and the Medical Department of the Mount Sinai Hospital, Service of Dr. George Baehr.

1. Shattuck, H. F.; Brown, J. C., and Preston, M. P.: Clinical Value of Some Recent Tests for Liver Function, Am. J. M. Sc. **170**:510, 1925. Murphy, W. P.: Biliary System Function Tests, Arch. Int. Med. **37**:797 (June) 1926; Boston M. & S. J. **194**:297, 1926. Piersol, G. M., and Rothman, M. M.: Practical Value of Liver Function Tests, J. A. M. A. **91**:1768 (Dec. 8) 1928. Cornell, V. H.: The Bromsulphalein Hepatic Function Test, Arch. Int. Med. **44**:818 (Dec.) 1929. Foley, E. F.: The Clinical Value of Tests of Liver Function, Arch. Int. Med. **45**:302 (Feb.) 1930. Mann, F. C., and Bollman, J. L.: Liver Function Tests, Arch. Path. **1**:681 (May) 1926.

2. Brugsch, T., and Horsters, H.: Cholerese und Choleretica: Ein Beitrag zur Physiologie der Galle, Ztschr. f. d. ges. exper. Med. **38**:367, 1923; II. Hypo- und Hypercholeresen, ibid. **43**:517, 1924; III. Ueber die choleretische Wirkung von Chinolinderivaten, insbesondere der Atophanreihe, ibid. **43**:717, 1924.

3. Skorczewski, W., and Sohn, I.: Ueber einige im Atophanharne auftretende charackteristische Reaktionen, Wien. klin. Wchnschr. **24**:1700 (Dec.) 1911.

tion of this compound in urine, which makes it possible to compare this phase of the metabolism of cinchophen in normal subjects and in those presenting definite or suspected evidence of hepatic dysfunction. Observations with this method indicate that in disturbances of the liver a larger percentage of the ingested drug appears in the urine in the form of oxy-cinchophen.[4] The slight oxidation of cinchophen to oxy-cinchophen is apparently carried on even by the diseased cells of the liver.

THE COLORIMETRIC ESTIMATION OF 2-(ORTHO-HYDROXY)-PHENYL-QUINOLINE-4-CARBOXYLIC ACID IN URINE

This method for estimating this substance in small quantities of urine was devised in order to replace the present laborious and less sensitive methods of chemical isolation.[5] It is based on a characteristic stable siskin-yellow color reaction[6] obtained when a few drops of urine containing oxy-cinchophen are added to concentrated hydrochloric acid.

Preparation of Standard Solutions.—A stock solution of 0.2 per cent oxy-cinchophen[7] in 5 per cent solution of sodium carbonate is prepared, from which are made standard solutions ranging from 0.0002 to 0.008 per cent oxy-cinchophen in concentrated iron-free hydrochloric acid.[8] Such solutions, hermetically sealed in glass tubes approximately 15 mm. in diameter, have thus far not faded in a period of fifteen months.

4. In the text the substance 2-(ortho-hydroxy)-phenyl-quinoline-4-carboxylic acid will be referred to as oxy-cinchophen for the sake of brevity.

5. Skorczewski, W., and Sohn, I.: Ueber das Verhalten des Atophans im Organismus, Wien. klin. Wchnschr. **25**:593 (April) 1912; Stoffwechselversuche bei Atophandarreichung, Ztschr. f. exper. Path. u. Therap. **11**:254, 1912. Dohrn, M.: Ueber das Verhalten des Atophans im Organismus, Biochem. Ztschr. **43**:240, 1912. Rotter, L.: Zur Kenntnis des Atophans und einiger Atophan Derivate, Ztschr. f. exper. Path. u. Therap. **19**:176, 1917. Fürth, O., and Kuh, E.: Biochemical Studies on the Absorption of the Ethylester of Paramethylphenylcinchoninic acid (Tolysin) and its Fate in Metabolism: I. The Absorption of Tolysin from the Intestinal Tract, J. Pharmacol. & Exper. Therap. **38**:57 (Jan.) 1930.

6. This color is described (footnote 3) as "Zeisig-Gelb." The siskin (G. Zeisig) is a small sharp-billed, chiefly greenish and yellowish finch (Spinus spinus) found in the temperate zone in Europe and Asia, allied to the goldfinch.

7. A lot of 2-(ortho-hydroxy)-phenyl-quinoline-4-carboxylic acid was synthesized for me by the Calco Chemical Company, Boundbrook, N. J. It is an orange or yellowish powder, the melting point being 242 C., and it is insoluble in petroleum ether, only slightly soluble in water and benzene and alcohol and more soluble in ether and methyl alcohol.

8. Chemically pure concentrated hydrochloric acid containing traces, or approximately 0.00005 per cent of iron may be employed for the test.

Sensitivity of the Test.—By this method oxy-cinchophen can be detected in a concentration of 1 : 500,000, or 0.0002 per cent. In lesser concentrations, the reaction with concentrated hydrochloric acid fails to give the characteristic yellow. Solutions of 0.0002 per cent of oxy-cinchophen in distilled water are colorless.

Colorimetric Properties of the Reaction.—The intensity of color of oxy-cinchophen reacting with concentrated hydrochloric acid was found to be directly proportional to the concentration of oxy-cinchophen in the range studied, between 0.04 per cent and 0.0002 per cent. This was established by comparing serial dilutions of oxy-cinchophen with permanent standard solutions. The color reaches its maximum almost immediately, and it is stable to heat. The intensity of color formation is governed by the concentration of hydrochloric acid. A minimal concentration of 0.0002 per cent of oxy-cinchophen fails to react with less than 5 per cent of hydrochloric acid. Dilutions of hydrochloric acid below 30 per cent result in a slight loss of intensity of color. Thus a maximum of 0.8 cc. of urine may be added to a final volume of 5 cc. of concentrated hydrochloric acid without loss of intensity of color due to the dilution of acid. In this concentration of urine the acid suffices to bleach the urochromes so that they are not an interfering color factor.

Atypical Reactions.—The chromogen identified by Herter as indolacetic acid,[9] which is present in many specimens of urine, yields urorosein, a rose pink substance, when treated with mineral acid. This color is unstable, and its formation is accelerated by heat. The excretion of this chromogen has been observed to vary in fractional specimens of urine. Sometimes it ceases to be excreted entirely. When it is present, a urine obtained prior to the ingestion of cinchophen serves as a control. Specimens of urine treated with concentrated hydrochloric acid may also give a carmine color.[9a]

The addition of bile to concentrated hydrochloric acid results in colors ranging from orange to green, the transition being accelerated by heat. When bile is present in urine, a specimen obtained prior to the intake of cinchophen serves as a control.

The presence of blood in the urine gives a faint yellow reaction probably due to the formation of acid-hematin. This interferes with the

9. Herter, C. A.: On Indolacetic Acid as the Chromogen of the "Urorosein" of the Urine, J. Biol. Chem. **4**:253, 1908.

9a. Fearon, W. R., and Thompson, A. G.: The Urocarmine Reaction, Biochem. J. **24**:1371, 1930. These authors apply the term urocarmine to a class of indogenide pigments obtained from the condensation of indoxyl with various phenols unsubstituted in the para-position. They believe the urocarmine reaction to be more common than the urorosein reaction. (Personal communication.)

colorimetric determination of oxy-cinchophen. Among several hundred tests, an interfering yellow color reaction, probably due to the formation of the oxides of chlorine, was twice found in the control urine before the ingestion of cinchophen. In both instances, the interfering substances were not present on subsequent days.

No atypical or interfering color reactions are encountered in urine after the administration of acetylsalicylic acid, sodium salicylate or the ethylester of paramethyl-phenylcinchoninic acid (neocinchophen).

THE OPTIMUM TEST DOSE OF CINCHOPHEN

When a test dose of 0.10 or 0.45 Gm. of cinchophen is administered at intervals of two days (table 1), the fraction excreted each time as oxy-cinchophen is fairly constant in normal and abnormal cases. But when the dose of cinchophen is increased to 0.90 Gm., great variability in the relative quantity of oxy-cinchophen excreted on the different test days is observed in abnormal cases (cases 4 and 5); therefore 0.45 Gm. of cinchophen has been adopted as the maximum test dose. Table 2 illustrates the constancy of the excretion of oxy-cinchophen on repeated tests with this dose in five normal and ten abnormal patients.

TABLE 1.—*Determination of the Optimum Test Dose of Cinchophen: Tests Repeated at Intervals of Two Days*

Case	Test No.	Excretion of Oxy-Cinchophen in Twenty-Four Hours, Mg.		
		100 Mg. Cinchophen	450 Mg. Cinchophen	900 Mg. Cinchophen
1	1	9	84	154
	2	11	75	146
2	1	20	90	185
	2	24	87	167
3	1	16	60	117
	2	20	65	146
4	1	30	110	158
	2	28	102	307
5	1	35	150	325
	2	40	178	202

FACTORS INFLUENCING QUANTITATIVE RESULTS

In order to define the conditions of the test, a study was made of factors that might influence the oxidation of cinchophen and the excretion of oxy-cinchophen.

Body Weight.—The oxidation of the test dose of cinchophen is independent of body weight within the range of from 44 to 90 Kg. in a series of normal subjects (table 3) and within a range of from 31.5 to 88 Kg. in a series of abnormal subjects (tables 4 to 10).

Diet.—The excretion of oxy-cinchophen is not appreciably influenced by diet. Some of the patients were observed while on a mixed

hospital diet; others were observed while on a diet high in carbohydrates (500 Gm. per day). Excessive intake of water may result in a diminished excretion of oxy-cinchophen probably due to a decreased rate of absorption from the bowel.

Medication.—Morphine and codeine, as well as cathartics, seem to decrease the excretion of oxy-cinchophen, probably by interfering with the absorption of cinchophen from the bowel. The actions of insulin and epinephrine modify the results of the test; the former by decreasing the excretion of oxy-cinchophen, the latter by sometimes increasing it. The simultaneous administration of sodium salicylate, acetylsalicylic acid or phenyl-ethyl-barbituric acid in therapeutic doses does not alter quantitative results.

TABLE 2.—*Comparison of Quantitative Results in Repeated Tests; Standard Oral Dose of 0.45 Gm.*

Case	Test No.	Date of Test	Excretion of Oxy-Cinchophen in 24 Hr., Mg.	Case	Test No.	Date of Test	Excretion of Oxy-Cinchophen in 24 Hr., Mg.
1	1	1/25/30	31	8	1	4/30/30	150
	2	2/ 7/30	50		2	5/ 2/30	178
2	1	3/28/30	76	9	1	1/27/30	167
	2	4/16/30	58		2	2/ 1/30	129
3	1	2/20/30	84	10	1	3/25/30	154
	2	3/11/30	65		2	3/28/30	186
	3	3/15/30	68	11	1	2/13/30	176
	4	3/17/30	68		2	2/17/30	192
4	1	2/19/30	68		3	2/23/30	207
	2	3/ 2/30	36	12	1	5/13/30	130
5	1	3/ 1/30	75		2	5/17/30	163
	2	3/ 7/30	81	13	1	5/ 9/30	375
6	1	4/30/30	75		2	5/15/30	353
	2	5/ 2/30	84	14	1	2/14/30	162
7	1	5/ 1/30	110		2	2/22/30	176
	2	5/ 2/30	102	15	1	5/ 9/30	118
					2	5/17/30	101

Extrahepatic Disease.—Marked impairment of renal function interferes with the excretion of oxy-cinchophen. Lesser degrees of renal impairment delay the excretion of oxy-cinchophen but do not appreciably influence the amount excreted over a period of twenty-four hours.

High intestinal obstruction interferes with the absorption of cinchophen. Malnutrition of long standing interferes with the accuracy of the test, possibly also because of diminished absorption of the drug. Drainage of ascitic fluid through the wound of an external abdominal paracentesis results in a diminished excretion of oxy-cinchophen.

Body Temperature.—The excretion of oxy-cinchophen bears no apparent relationship to the body temperature at the time of the test (a range of from 98 to 104.2 F. was studied—tables 4 to 9).

Routes of Administration.—A comparison between the oral and the intramuscular routes of administration of cinchophen was made. For the latter, a standard dose of 0.45 Gm. of cinchophen sodium was employed. The intramuscular method gave a larger excretion of oxy-cinchophen in seven of ten cases and a lesser excretion in three cases. Repeated intramuscular tests on the same subject did not give uniform results. The inconsistency of the results obtained by the intramuscular route are probably due to an inconstant rate of absorption from the site of injection.

Antemortem Determinations.—Tests made one, two or three days before death were usually found to give a definitely diminished excretion of oxy-cinchophen. Under these conditions the normal as well as the markedly diseased cells of the liver may lose even the capacity to convert cinchophen to oxy-cinchophen.

SITE OF OXIDATION OF CINCHOPHEN

It is generally assumed that cinchophen is oxidized by the cells of the liver, although no adequate proof has been presented. Rotter [10] found no evidence of oxidation of cinchophen by the dog's liver and suggested that the liver of animals was different in this respect from that of human beings. In the present research her work was repeated with modifications, and negative results were also obtained.

These experiments consisted in testing colorimetrically the filtrates of suspensions of cells of crushed muscle, lung, liver, kidney, spleen, heart and intestine of freshly killed rabbits that had been receiving intramuscular injections of cinchophen and were excreting oxy-cinchophen at the time they were killed. Similar suspensions of cells were also incubated at 37 C. and aerated, after the addition, in vitro, of a buffered solution of cinchophen. However, hemolyzed blood contained in the control filtrates of the lung and liver gave interfering color reactions.

Indirect evidence that the liver is the site of oxidation of cinchophen in human subjects was obtained by comparing the time of appearance and the concentration of oxy-cinchophen in the urine and in bile obtained by transduodenal drainage. Bile aspirated before the intramuscular administration of cinchophen served as a control. The procedure for the detection of oxy-cinchophen in bile is the same as that for urine. In four subjects observed, oxy-cinchophen appeared in the bile from one-half to one hour before it appeared in the urine. In one instance it appeared in the bile at the end of one and a half hours, and in the other three subjects it appeared at the end of two hours. In two

10. Rotter (footnote 5, third reference).

instances it was found in the same concentration in the bile as in the urine and in the other two in higher concentrations in the bile. It appears that oxy-cinchophen is excreted earlier in the bile than in urine and sometimes in higher concentrations.

Oxy-cinchophen could not be demonstrated by my method in the blood plasma following the standard dose given orally, although when it was added to blood plasma it could be detected in the same concentrations as in urine. Therefore, there is no appreciable threshold of excretion of this substance.

Additional evidence that the liver cells are responsible for the conversion of cinchophen to oxy-cinchophen was obtained by the following experiment: In two rabbits under ether narcosis, all of the vessels to the liver and the common bile duct were clamped, and then 2 cc. of cinchophen sodium was given intravenously. They survived two hours and did not show the slightest trace of oxy-cinchophen in the urine. Two control rabbits, similarly treated, save that in one the vessels to the liver were not occluded, and in the other only the vessels to the left lobe were clamped, showed traces of oxy-cinchophen in the urine at the end of two hours.

HEPATOTOXIC ACTION OF CINCHOPHEN

Objection may be raised to the use of cinchophen as a test substance in patients with hepatic disorders in view of the recent reports of damage to the liver ascribed to its administration in therapeutic doses.[11] There is evidence, however, that a single or even repeated standard test doses of 0.45 Gm. are not toxic, even in the presence of severe damage of the liver. Studies were made on a patient with degeneration of the liver attributed to the ingestion some time before of approximately 18 Gm. of cinchophen in a period of eighteen days. During the period of severe jaundice, fever and drowsiness, with an icteric index of 100, the test showed an excretion of 375 mg. of oxy-cinchophen on the test dose of 0.45 Gm., the maximum excretion thus far obtained in these studies. One week later, with slight clinical improvement, the excretion was 353 mg. in twenty-four hours; sixteen days later, with marked clinical improvement, 141 mg. As will be demonstrated in cases of disease of the liver, excretions above 300 mg. signify severe damage of the liver. Thus in this case ascribed to the hepatotoxic action of cinchophen, three doses of 0.45 Gm. of this substance given at intervals of approximately a week had no toxic action and did not

11. Reichle, H. S.: Toxic Cirrhosis of Liver Due to Cinchophen, Arch. Int. Med. **44**:281 (Aug.) 1929. Rabinowitz, M. A.: Atrophy of the Liver Due to Cinchophen Preparations, J. A. M. A. **95**:1228 (Oct. 25) 1930.

delay clinical and functional recovery. However, I do not advise the performance of the tests in this type of case.

Similar observations have been made in all the cases of disease of the liver that have been studied. Thus far no instance of untoward effects attributable to the cinchophen employed in the test has been encountered. When the disturbance of the liver is due to a relatively benign process, such as in the mild forms of so-called catarrhal jaundice, resolution takes place simultaneously with the administration of repeated test doses of cinchophen. In fact, in many instances a decrease in the intensity of the jaundice is noted on the day following the test, which may be due to the choleretic action of the drug.

Further evidence of the nontoxicity of the test dose of cinchophen was obtained by a comparison of the icteric index before, during and after its administration. Four patients were studied, of whom three had latent jaundice. The first, with a normal index (4), showed no change during or after the administration of two doses of 0.45 Gm.; after the administration of two further doses of 0.9 Gm. each, the index was 5 and 6, respectively. The second case (duodenal ulcer), with an initial icteric index of 10, showed a slight rise to 12 after a single dose of 0.45 Gm. Twenty-four hours after a total of 2.7 Gm. of cinchophen had been given within seven days, the icteric index had dropped to 8. In the two remaining cases (pneumonia and cirrhosis of the liver) the initial indexes of 10 rose to 15 and 20, respectively, after the test dose, but a prompt return to indexes even below the original levels (5 and 9, respectively) was noted. The temporary rises observed after the test dose may possibly be physiologic, for the same phenomenon has been observed in normal persons under the stimulation of a test for dextrose tolerance.[12] A similar slight temporary increase in serum bilirubin followed by a rapid decline was noted by others following the administration of cinchophen in the treatment for catarrhal jaundice and for icterus due to cystadenoma of the bile duct.[13] The prompt return to the original index following the administration of two test doses and two larger doses, 2.75 Gm. of cinchophen in all, over a period of seven days encourages the belief that the excretory function of the liver is not impaired by the procedure.

A study of the amino-acid content of the blood plasma in one normal patient and one abnormal patient before and immediately following a test dose of cinchophen revealed no change in the deamination

12. Bernheim, A. R.: The Significance of Variations of Bilirubinemia, Arch. Path. **1**:747 (May) 1926.

13. Grünenberg, K., and Ullmann, H.: Atophan Wirkung bei Erkrankungen der Leber und der Gallenwege, Med. Klin. **20**:663 (May 18) 1924.

function of the liver. The Folin colorimetric method [14] was employed; the test was made in duplicate, and the average results were recorded. In the normal case, the amino-acid nitrogen content of the blood was 5.1 mg. per hundred cubic centimeters before the administration of cinchophen and 5 afterward; in the abnormal case, 6.35 mg. before and 4.9 after.

Experimentation on animals also failed to reveal any hepatotoxic effect of cinchophen. Six rabbits weighing approximately 2 Kg. each, receiving 0.45 Gm. of cinchophen sodium intramuscularly daily over a period of three weeks, with or without daily doses of 0.5 cc. of epinephrine administered subcutaneously, maintained their appetite and weight and showed no gross evidence of degeneration of the liver. Only those that were starved showed some loss in weight. The negative observations on animals, however, prove nothing regarding the susceptibility of human beings to cinchophen.

It is of interest to note that the therapeutic use of cinchophen has been recommended in cases of hepatic disease with jaundice.[15]

THE FATE OF CINCHOPHEN IN THE ORGANISM

It was known to the original investigators of the actions of this drug [16] that it is readily destroyed in the organism. Following the administration of 3 Gm. of cinchophen daily for three days to a human being, they found that approximately 5.5 per cent reappeared in the urine unchanged. By improved chemical methods, the absorption of cinchophen from the bowel of the dog has been found to be practically a quantitative one.[17] The principal conversion product of the metabolism of cinchophen following large doses of the drug is 2-(orthohydroxy)-phenyl-quinoline-4-carboxylic acid (oxy-cinchophen). Rotter [17a] isolated in substance 0.2732, 1.515 and 1.484 Gm., respectively, of this substance from the urine of three patients who had each ingested 2 Gm. of cinchophen in doses of 1 Gm., an hour apart. Skorczewski and Sohn [18] stated that following the prolonged administration of

14. Folin, O.: A New Colorimetric Method for the Determination of the Amino-Acid Nitrogen in Blood, J. Biol. Chem. **51**:377, 1922.

15. Umber, F., in Mohr, L., and Staehlin, R.: Erkrankungen der Leber: Handbuch der innere Medizin, ed. 2, Berlin, Julius Springer, 1926, p. 1. Slobozianu, H.: Die Behandlung des Ikterus der Neugeborenen, Arch. f. Kinderh. **77**:58 (Dec.) 1925.

16. Nicolaier, A., and Dohrn, M.: Ueber die Wirkung von Chinolincarbonsäure und ihre Derivate auf die Ausscheidung der Harnsaure, Arch. f. klin. Med. **93**:331, 1908.

17. Fürth and Kuh (footnote 5, fourth reference).

17a. Rotter, L.: Zur Kenntnis des Atophans und einiger Atophan Derivate, Ztschr. f. exper. Path. u. Therap. **19**:176, 1917.

18. Skorczewski and Sohn (footnote 5, first reference).

cinchophen, oxy-cinchophen disappears from the urine. Under such conditions, the catabolism of cinchophen is probably altered.[19]

The author compared the daily excretion of oxy-cinchophen in the urine following the ingestion of 0.1 Gm. of cinchophen, and 0.1 Gm. of oxy-cinchophen, respectively, in four normal subjects and found that the excretion was approximately the same for both; the observations were somewhat higher in all instances with the latter substance. In three cases of disease of the liver, a comparison showed that there was an increase in the excretion of oxy-cinchophen following the ingestion of 0.1 Gm. of oxy-cinchophen corresponding to that found following the ingestion of the same amount of cinchophen. This would indicate that the increase in the excretion of oxy-cinchophen encountered in impairment of hepatic function is probably due to interference in the catabolism of cinchophen beyond the state of oxy-cinchophen. The preliminary slight oxidation of cinchophen to oxy-cinchophen is apparently carried on even by the diseased cells of the liver.

CLINICAL APPLICATION OF THE TEST FOR HEPATIC FUNCTION

Collection of Urine.—The routine procedure for the performance of the test in hospital practice is as follows: A routine specimen is obtained at 5 a. m. At 6 a. m., 0.45 Gm. of cinchophen contained in a pharmaceutic gelatin capsule is administered to the patient. The patient is given his usual diet, and only a moderate amount of fluids is allowed. Fractional quantitative specimens are obtained at 10 a. m., 12 a. m., 2 p. m., 4 p. m., 6 p. m., 10 p. m., and all the urine between 10 p. m. and 6 a. m. the following morning is saved as a final specimen. Random voidings are also saved. When accurate measurement of the volumes of the fractional specimens can be depended on, only samples need be saved in ordinary test tubes. During the test the patient should receive no morphine, codeine, insulin, epinephrine, cathartic or enema, or any medication that might affect the absorption or oxidation of cinchophen. For the correct interpretation of the test it is essential that the urine be collected quantitatively over the twenty-four hour period and that the patient receive and retain the standard dose. Vomiting vitiates the results. When the patient is unable to void at the specified hour, a statement to that effect is recorded. The determination is best made on fractional specimens rather than on a pooled twenty-four hour specimen, because dilution may interfere with the detection of the substance.

Colorimetric Estimation of Oxy-Cinchophen.—If the urine contains bile or albumin, from 5 to 10 cc. of each specimen is heated to boiling and filtered. In the absence of bile or albumin, the samples need only to be filtered; 0.2 cc. of each filtrate is then added to concentrated hydrochloric acid to make a total volume of 5 cc.; the mixture is shaken and brought to a boil. If the yellow color of the reaction does not develop, 0.4, 0.6 or 0.8 cc., respectively, of urine is added to concentrated hydrochloric acid to make a final volume of 5 cc. and similarly treated.

19. For further oxidation products of cinchophen excreted in the urine, see Dohrn (footnote 5, second reference), or produced in vitro, see Böhm, R., and Bournot, K.: Ueber die Phenyl-chinolin-4-carbonsaure (Atophan) und ihre Oxydationsprodukte, Ber. d. deutsch. chem. Gesellsch. **48**:1570 (Sept.) 1915.

The control urine, obtained at 5 a. m., prior to the administration of cinchophen, is treated identically.

It is sometimes necessary to prepare several control tubes with the aforementioned proportions of urine in order to match the test solution, owing to the fact that the concentrations of the indolacetic acid or bile responsible for atypical reactions vary in the fractional specimens.

The heated mixtures are allowed to stand for approximately thirty minutes before reading by comparison with permanent standard solutions in a comparator block fitted for four tubes, approximately 15 mm. in diameter, in two rows, and viewed against diffuse daylight through ground glass. The control is placed behind the standard solution and a tube of concentrated hydrochloric acid behind the test solution. When a test solution directly matches any of the standards, the control tube is not placed behind the standard. In the presence of bile, better comparison of color is often obtained when only 0.1 cc. of urine is added to a final volume of 5 cc. of hydrochloric acid.

The presence of blood in the urine interferes with the test owing to the development of the yellow color of acid-hematin when added to hydrochloric acid.

Calculation.—The percentage of oxy-cinchophen determined colorimetrically represents the concentration of this substance in 0.1, 0.2, 0.4, 0.6 and 0.8 cc. of urine, respectively, diluted to a final volume of 5 cc. It must be multiplied by the respective dilutions, 50, 25, 12.5, 8.33 or 6.25 to obtain the percentage of oxy-cinchophen in the original urine. The result for twenty-four hours is expressed in milligrams as well as in the percentage of the test dose excreted as oxy-cinchophen. This percentage is calculated on the basis of a theoretical conversion of 450 mg. of cinchophen into 480 mg. of oxy-cinchophen.

Repetition of the Test.—When the test is to be repeated for confirmation or in order to observe the progress of a case, it is advisable to allow at least one day between tests.

QUALITATIVE AND QUANTITATIVE STUDIES ON THE EXCRETION OF OXY-CINCHOPHEN IN SUBJECTS WITHOUT HEPATIC DYSFUNCTION

The time of appearance and disappearance of oxy-cinchophen in the urine following the ingestion of a standard dose of 0.45 Gm. of cinchophen was studied in twenty-five subjects over a period of thirty-six hours. Fractional specimens were collected every hour during the first six hours, every two hours in the next six hours, and single specimens at the end of eighteen, twenty-four, thirty and thirty-six hours, respectively. Oxy-cinchophen appeared in the urine in the second or third hour. Its excretion was completed in all cases by the twenty-fourth hour, in the majority of cases by the eighteenth hour and in many cases at the end of twelve hours. In only two cases was a trace of oxy-cinchophen found in the urine voided at the end of twenty-four hours.

The amount of oxy-cinchophen excreted in the urine after the ingestion of a standard dose of 0.45 Gm. of cinchophen was determined in a series of fifty selected normal patients (table 3). The quantities of oxy-cinchophen excreted over a period of twenty-four hours varied

TABLE 3.—*Excretion of Oxy-Cinchophen in Urine in Twenty-Four Hours in Fifty Selected Cases Without Hepatic Dysfunction; Test Dose of 0.45 Gm. of Cinchophen; Rate of Excretion; Relation of Excretion to Body Weight*

Case	Body Weight, Kg.	Oxy-Cinchophen in Fractional Specimens, per Cent	Excretion of Oxy-Cinchophen in Second 12 Hours, Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
				Mg.	Per Cent	
1	57	0.0088-0.0250	0	53	14.0	Bronchopneumonia, bilateral: convalescent
2	72	0.0044-0.0088	0	88	18.0	Syphilis; stricture of rectum
3	?	0.0025-0.0125	5	63	13.0	Chyluria
4	44	0.0050-0.0250	0	81	17.0	Neoplasm of pharynx, pituitary gland and stomach
5	65	0.0040-0.0250	0	90	19.0	Polyarthritis (nonrheumatic)
6	52	0.0025-0.0125	45	97	20.0	Mitral stenosis and insufficiency
7	85	0.0044-0.0250	13	55	11.0	Obesity; hypertension
8	44	0.0050-0.0200	45	88	18.0	Lobar pneumonia (convalescent); pulmonary tuberculosis
9	50	0.0100-0.0175	0	84	17.5	Atherosclerotic heart disease
10	80	0.0025-0.0063	9	59	12.0	Bronchopneumonia (convalescent); hypertension
11	56	0.0025-0.0044	0	31	6.5	Secondary anemia
12	56	0.0025-0.0250	28	90	19.0	Typhus fever (convalescent)
13	70	0.0044-0.0088	36	68	14.0	Coronary artery disease; paronychia
14	55	0.0025-0.0050	24	58	12.0	Subacute bacterial endocarditis
15	?	0.0050-0.0125	9	70	14.5	Subacute bacterial endocarditis
16	52	0.0050-0.0125	0	57	12.0	Infectious arthritis; tonsillitis
17	83	0.0063-0.0125	0	71	15.0	Essential thrombocytopenic purpura
18	53	0.0050-0.0250	0	87	18.0	Mitral stenosis and insufficiency
19	57	0.0025-0.0125	0	77	16.0	Erythema nodosum; tonsillitis
20	74	0.0044-0.0125	0	47	10.0	Coronary sclerosis
21	71	0.0025-0.0088	23	88	18.0	Coronary artery thrombosis
22	77	0.0025-0.0125	0	44	9.0	Benign neoplasm of bronchus
23	59	0.0050-0.0125	26	63	13.0	Hypertension; atherosclerosis
24	75.5	0.0050-0.0088	?	73	15.0	Meningococcemia; chronic arthritis
25	79	0.0088-0.0175	0	43	9.0	Postoperative hernia
26	89	0.0088-0.0250	0	91	19.0	Obesity; hypertension
27	58	0.0044-0.0250	13	82	17.0	Lobar pneumonia (convalescent)
28	61	0.0044-0.0125	24	85	17.5	Erythema nodosum
29	61	0.0050-0.0500	26	80	17.0	Hypertension; atherosclerosis
30	73	0.0125-0.0125	0	47	16.0	Coronary artery thrombosis
31	76	0.0025-0.0088	18	60	12.5	Hypertension; diabetes
32	50	0.0175-0.0500	0	90	19.0	Diabetes mellitus; coronary artery disease
33	?	0.0125-0.0250	0	91	19.0	Sinus following empyema of thorax
34	44	0.0050-0.0125	18	61	12.5	Aortic insufficiency; nasopharyngitis
35	66	0.0063-0.0125	0	52	11.0	Secondary anemia
36	59	0.0044-0.0500	30	60	12.5	Hyperthyroidism; hypertension
37	66	0.0016-0.0500	0	74	15.5	Delirium tremens; chronic alcoholism
38	50	0.0050 0.0125	24	54	11.0	Hypertension; coronary artery disease; secondary anemia
39	90	0.0040-0.0088	16	60	12.5	Syphilis; hematemesis
40	?	0.0050-0.0250	0	64	13.5	Psychoneurosis
41	50	0.0044-0.0050	45	90	19.6	Carcinoma of nasopharynx
42	50	0.0050-0.0250	0	61	12.5	Secondary anemia
43	70	0.0025-0.0200	23	84	17.5	Coronary thrombosis
44	56	0.0025-0.0200	0	95	20.0	Gastric ulcer; hour-glass stomach
45	51	0.0050-0.0250	24	84	17.5	Perisigmoiditis
46	68	0.0050-0.0050	48	78	16.0	Angina pectoris; herpes zoster
47	56	0.0050-0.0375	0	79	16.5	Neurocirculatory asthenia
48	58	0.0025-0.0125	23	84	17.5	Essential hypertension; herpes zoster
49	56	0.0050-0.0000	40	53	11.0	Carcinoma of pharynx
50	60	0.0050-0.0375	0	88	18.0	Acute aseptic lymphocytic meningitis

between 30 and 100 mg., which represents approximately from 7 to 21 per cent of the test dose. The concentration of oxy-cinchophen in fractional specimens of urine varied between 0.0013 and 0.025 per cent (in four cases). In all but two cases the major amount was excreted in the first twelve hour period. In about one half of the cases there was no excretion of oxy-cinchophen in the second twelve hour period. In one half of the remaining cases less than one third of the total amount was excreted in the second twelve hour period.

QUALITATIVE AND QUANTITATIVE STUDIES ON THE EXCRETION OF OXY-CINCHOPHEN BY PATIENTS WITH HEPATIC DYSFUNCTION

The amount of oxy-cinchophen excreted in the urine after the ingestion of a standard dose of 0.45 Gm. of cinchophen was determined in a series of 100 selected patients with hepatic dysfunction (tables 4 to 10).[20] The quantities of oxy-cinchophen excreted over a period of twenty-four hours varied between 100 and 375 mg., which represents approximately from 21 to 78 per cent of the test dose. The concentration of oxy-cinchophen in fractional specimens of urine varied between 0.0013 and 0.1 per cent. In nine instances a major amount was excreted in the second twelve hour period. In slightly more than one half of the cases less than one third of the total excretion occurred in the second twelve hour period.

Compared to the series of normal patients, the patients with pathologic conditions show a definitely greater total excretion, at times a higher concentration in fractional specimens and a tendency to a more prolonged excretion of oxy-cinchophen.

Although oxy-cinchophen first appeared in the urine in the second rather than in the third hour more often in the pathologic cases than in the normal subjects, this observation was not proportional to the severity of the functional disturbance and seemed to be of no diagnostic import. In two patients with mild functional disturbance, excretion was observed within the first hour.

The maximum excretion per hour both by normal subjects and by persons with pathologic conditions occurred between the sixth to the tenth hour after the intake of cinchophen. This coincides with the period following the midday meal and may be of physiologic significance.

When the progress of the disease seemed to be clinically stationary, repeated tests gave almost the same figures. Whenever the disturbance of function of the liver was clinically progressive or regressive, corre-

20. Dr. Leo Kessel and Dr. Richard Lewisohn gave me permission to study some of these cases from their respective services.

sponding results were usually obtained that gave a fairly accurate indication of the increase in severity or of improvement.

These observations indicate that the excretion of oxy-cinchophen after the administration of a test dose of 0.45 Gm. of cinchophen may be used as an index of hepatic function. The results of the test may be expressed in milligrams excreted or in percentage of the test dose excreted in the urine as oxy-cinchophen. Thus an excretion of more than 100 mg., or 21 per cent of the test dose, indicates disturbed function of the liver.

The results of the application of this test in various diseases and functional disturbances of the liver will next be presented.

DISEASE OF THE GALLBLADDER AND BILE PASSAGES

The results tabulated in table 4 indicate that cases of acute and chronic inflammation of the gallbladder, with or without obstruction of the common duct due to stone or stricture, usually show relatively slight disturbance of the function of the liver, as manifested by figures above 100 and less than 200 mg., or from 21 to 42 per cent. The exceptions to the rule were the patient in case 3 (chronic cholecystitis and cholelithiasis) with a normal observation and the one in case 15 in which a man with a huge solitary stone of the common duct and slight fever showed excretions of 229 mg. (47.5 per cent) and 304 mg. (63 per cent). While the latter case gave no clinical indication of severe disturbance of the function of the liver, the icteric index was 50, and the bromsulphalein test showed 25 per cent retention in thirty minutes, thereby confirming the significance of the test.

The relief from biliary obstruction has a tendency to lower the figures. During the three to four weeks after operation that I have been able to follow up such cases, there usually has not been a return to normal. The presence or absence of fever does not seem to affect the results materially. The presence of cholangeitis, at least of the larger bile ducts, does not appear to disturb this function of the liver cell to any great extent. Cases 6 and 10, in which there was fever up to 104 F., terminated fatally, and showed the presence of suppurative cholangeitis post mortem, yet the test gave practically the same results as in other cases of disease of the common duct not complicated by suppuration.

CIRRHOSIS OF THE LIVER

Thirteen cases of cirrhosis of the liver due to various causes were studied (table 5). Three gave normal results (cases 20, 21 and 22). Case 18 was that of a woman in whom acute damage of the liver developed following an alcoholic spree. It is interesting that while

TABLE 4.—*Excretion of Oxy Cinchophen in Disease of the Gallbladder and Bile Passages: Test Dose of 0.45 Gm. of Cinchophen; Rate of Excretion; Relation of Excretion to Body Weight and Temperature*

Case	Weight, Kg.	Temperature, F.	Jaundice	Icterus Index	van den Bergh Reaction		Date of Test, 1930	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
					Direct	Quantitative, Mg.			Mg.	Per Cent	
1	....	101.0	0	..	Negative	0.5	2/12	27	171	35.5	Chronic cholecystitis; cholelithiasis (laparotomy)
2	....	100.0	0	8	Negative	0.2	1/21	0	138	28.5	Chronic cholecystitis; cholelithiasis
3	....	.....	+	..	.....	...	5/ 6	5	75	15.5	Chronic cholecystitis; cholelithiasis
4	....	100.2	0	..	Negative	1.0	5/10	20	127	26.5	Chronic cholecystitis; cholelithiasis
5	44.0	100.2	+	45	Negative	0.2	5/15	48	147	30.5	Chronic cholecystitis; cholelithiasis; choledocholithiasis
	45.0	99.0	+	..	.....	...	5/18	24	99	20.5	
6	....	102.6	+	45	Positive	1.5	4/27	85	175	36.5	Chronic cholecystitis; cholelithiasis; choledocholithiasis; cholangeitis (necropsy)
		104.0	+	36	Positive	2.0	5/11	54	106	22.0	
7	72.0	98.6	+	20	Positive	0.5	6/ 7	0	158	33.0	Chronic cholecystitis; cholelithiasis; choledocholithiasis
	73.0	98.6	+	18	.....	...	6/10	38	155	32.5	
	73.0	98.6	+	..	.....	...	6/15	58	165	34.5	
8	50.0	99.8	+	..	Hemolyzed	0.2	5/13	40	130	27.0	Cholelithiasis; choledocholithiasis
	50.5	99.2	+	18	.....	...	5/17	63	163	34.0	
9	77.0	99.4	+	65	Delayed	0.9	5/30	99	170	35.5	Cholelithiasis; choledocholithiasis
		99.4	+	50	Positive	...	6/ 2	27	97	20.0	
10	43.0	103.0	+	..	Positive	0.9	2/13	40	165+	34.5+	Cholecystitis; choledocholithiasis; purulent cholangeitis; biliary fistula (necropsy)
11	....	100.0	+	50	Negative	1.0	3/30	19	113	23.5	Cholecystitis; cholelithiasis; stone of common duct
12	....	99.2	+	..	Negative	0.3	2/13	35	154	32.0	Choledocholithiasis
13	60.0	99.8	+	20	Prompt positive	2.0	4/11	0	129	27.0	Choledocholithiasis; cholelithiasis
14	54.0	99.0	0	30	Negative	0.2	2/18	0	150	31.5	Stricture of common duct; duodenal fistula (laparotomy)
15	61.0	99.6	+	50	Delayed	0.7	7/ 1	48	229	47.5	Stone of common duct (laparotomy)
	61.0	100.0	+	..	Positive	...	7/ 4	34	304	63.0	
16	61.0	99.2	+	36	Positive	1.5	6/16	26	154	32.0	Acute cholecystitis; cholangeitis; "infectious jaundice"
	61.0	99.4	+	15	Positive	0.5	6/19	39	168	35.0	

TABLE 5.—*Cirrhosis of the Liver*

Case	Weight, Kg.	Temperature, F.	White Blood Cells	Jaundice	Icterus Index	van den Bergh Reaction: Direct	van den Bergh Reaction: Quantitative, Mg.	Retention of Bromsulphalein, per Cent in 30 Minutes	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours: Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours: Per Cent	Diagnosis
17	58.5	100.2	9,100	+	10	Delayed	1.2	45	1/19	50	300	62.5	Biliary cirrhosis (biopsy)
	57.5	100.0	.....	+	15	Positive	...	..	2/ 7	98	349	73.0	
	57.5	99.8	.....	0	15	.....	...	..	2/20	45	113	25.0	
18	56.5	100.6	5,000	+	30	Delayed	1.0	..	2/ 6	30	106+	22.0+	Alcoholic cirrhosis
	56.5	99.2	.....	+	..	Positive	...	..	2/ 9	?	223+	46.5+	
	55.0	99.8	.....	Slight	25	.....	...	25	2/16	0	332	69.0	
19	95.0	99.6	8,000	0	10	Negative	0.2	5	4/29	0	204	42.5	Alcoholic cirrhosis
20	55.5	98.8	.....	0	30	Delayed	1.2	35	2/28	13	91	19.0	Alcoholic cirrhosis
	55.5	100.0	11,200	0	8	Positive	...	0	3/ 4	26	73	15.0	
21	41.0	99.6	.....	0	..	.....	...	..	2/27	9	65	13.5	Syphilitic cirrhosis (portal); laparotomy
22	56.0	98.6	10,400	0	8	Negative	0.2	5	3/ 6	0	74	15.5	Cirrhosis; splenic thrombosis
23	66.0	99.2	8,800	0	15	Delayed positive	0.6	20	2/25	16	109	22.5	Cirrhosis (portal, Laennec); ascites (necropsy)
24	50.0	99.2	9,200	+	125	Prompt	3.0	50	5/27	0	185	38.5	Syphilitic cirrhosis
	49.5	99.8	.....	+	75	Positive	...	..	5/29	66	106	22.0	
	49.5	99.4	.....	+	..	.....	...	..	6/ 1	0	66	14.0	
	50.0	99.0	.....	(receding)	..	.....	...	..	6/ 6	140	315	65.6	
	50.0	99.4	.....	+	120	.....	...	..	6/14	88	164	34.0	
25	52.0	99.0	6,100	+	125	Positive	5.5	..	2/14	24	250	52.0	Syphilitic cirrhosis
	53.0	99.6	.....	+	40	Delayed	0.3	..	2/16	0	116	24.0	
	53.0	99.0	.....	0	..	Positive	...	..	2/23	0	60	12.5	
26	65.0	99.6	5,550	+	30	Negative	1.0	5	5/ 3	15	142	29.5	Hepatosplenomegaly; hyperthyroidism
27	75.5	99.8	5,600	0	12	Negative	0.5	20	6/ 8	44	143	30.0	Splenomegalic cirrhosis; anemia (necropsy)
	75.0	99.4	.....	0	..	.....	...	..	6/11	25	100	21.0	
28	52.0	99.4	2,900	0	10	Negative	0.2	5	5/ 3	8	260	54.0	Hematosplenomegaly (schistosomiasis)
	52.0	99.4	.....	0	..	.....	...	..	6/ 7	0	143	30.0	
29	57.0	99.6	5,360	0		Delayed	0.8		5/29	21	137	28.5	Hematosplenomegaly (melanosis)
	57.0	99.2	.....	0		Positive	...		6/ 1	54	190	39.5	

the jaundice was receding and the patient was apparently improving clinically, successive tests showed increasing disturbance of the function of the liver. Case 17 illustrates the value of the test in following the course of pathologic changes. The patient had a huge liver which on biopsy showed hypertrophic biliary cirrhosis. Under observation the liver rapidly diminished in size until it was felt just below the costal margin. The successive observations showed a rapid fall in excretion from 300 and 349 mg. (62.5 and 73 per cent) to 113 mg. (23.5 per cent).

Results above 200 mg. (42 per cent) of excretion in cases of cirrhosis of the liver seem to signify the presence of acute degenerative changes; those between 150 mg. (31.5 per cent) and 200 mg. (42 per cent) suggests mild or receding degenerative changes. Normal results and figures below 150 mg. (31.5 per cent) were obtained in cases that were clinically "quiescent."

No parallelism was noted between the quantitative results of the van den Bergh test, the icteric index, the Rosenthal bromsulphalein test and the test with oxy-cinchophen in this group of cases. The reason for this probably lies in the fact that the first three tests are influenced by biliary obstruction, whereas the test with oxy-cinchophen is influenced only by parenchymal disease or other factors that disturb the function of the hepatic cells.

CATARRHAL JAUNDICE

The group comprises four cases of painless jaundice occurring in patients with no antecedent history indicative of disease of the liver or of the gallbladder, with and without fever and accompanied by gastro-intestinal disturbances such as vomiting and diarrhea (table 6). The results were the upper limit of normal or low abnormal figures, well below 200 mg. (42 per cent), such as are usually found in cholelithiasis and choledocholithiasis.

A significant observation is the sudden temporary increase in the excretion of oxy-cinchophen observed in cases 31 and 32, suggesting transitory damage of the liver and a rapid reparative process. An absolutely normal level was not reached, even when complete clinical recovery had apparently taken place. Case 30 showed the presence of tyrosine in the urine in a single examination by the tyrosinase method.[21]

In case 33, studied prior to a complete remission of the jaundice, the figures were only slightly elevated. Two months later jaundice recurred, and the patient died. Necropsy revealed subacute yellow

21. Lichtman, S. S., and Sobotka, H.: An Enzymatic Method for the Detection and Estimation of Tyrosine in Urine, J. Biol. Chem. **85**:261 (Dec.) 1929.

TABLE 6.—*Catarrhal Jaundice*

Case	Weight, Kg.	Temperature, F.	White Blood Cells	Jaundice	Icterus Index	van den Bergh Reaction: Direct	van den Bergh Reaction: Quantitative, Mg.	Retention of Bromsulphalein, per Cent in 30 Minutes	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours: Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours: Per Cent	Diagnosis
30	72.5	101.4	17,800	+	30	Negative	4.5	100	11/28/29	?	203+	42.0+	Catarrhal jaundice
31	63.5	98.6	5,100	+	..	Prompt, positive	0.3	..	2/ 4	6	113	23.5	Catarrhal jaundice
	63.0	99.0	.....	0	..	Delayed, positive	0.2	..	2/ 7	37	243	51.0	
	66.0	99.0	.....	0	..	.....	...	..	2/10	0	95	20.0	
32	59.0	99.2	7,800	+	70	Negative	0.5	60	4/ 8	0	96	20.0	Catarrhal jaundice
	63.0	99.0	.....	+	120	Prompt, positive	9.0	..	4/12	0	109	22.5	
	59.5	99.2	.....	+	75	Delayed, positive	0.3	..	4/18	0	204	42.5	
	61.5	99.2	.....	+	75	Delayed, positive	0.3	..	4/23	0	84	17.5	
	61.5	99.0	.....	0	20	Delayed, positive	0.2	..	4/29	127	357	74.0	
	63.0	99.0	.....	0	30	Delayed, positive	0.3	..	5/11	48	149	31.0	
	66.5	99.0	.....	0	..	.....	...	..	5/18	30	153	32.0	
33	79.0	99.4	7,000	+	150	Delayed, positive	1.5	..	5/ 9	48	118	24.5	Catarrhal jaundice (necropsy)
	80.0	100.8	.....	+	70 45	Delayed, positive	1.5	..	5/17	33	106	22.0	

atrophy of the liver. Unfortunately, because of vomiting a satisfactory test was not made in the patient's second admission to the hospital.

Although the figures for the excretion of oxy-cinchophen in catarrhal jaundice are often only slightly above normal, the transitory character of the rise at one stage of the disease in two of the cases suggested the presence of true parenchymal degeneration, from which recovery was apparently rapid. The occurrence of pathologic changes of this nature in cases that are clinical examples of catarrhal jaundice has already been recognized.[22]

"TOXIC HEPATITIS"; HEPATOSIS; CHRONIC HEPATITIS; "CHOLANGEITIS LENTA"

In this group are included eight cases of diffuse inflammatory or degenerative disease of the liver, with or without jaundice, sometimes associated with fever and chills and with or without enlargement of the liver. In some cases the condition is referred to as acute or chronic hepatitis. One is a case of so-called "cholangeitis lenta," in which there proved to be an inflammatory process involving the periportal fields in the vicinity of the bile ducts. Others are cases of "toxic hepatitis" or hepatosis due to intoxications with arsenicals, phenyl-cinchoninic acid or phenyl-ethyl barbituric acid, etc. (table 7).

Case 35 is an unusual instance of icterus due to degeneration of the liver that followed repeated therapeutic intravenous infusions of hypertonic salt solution (thrombo-angiitis obliterans). This case was studied in the convalescent stage and even then showed an excretion of 302 mg., or 63 per cent, with a rapid decline to 37 per cent in one week coincidental with improvement. Case 34 is an instance of icterus following the ingestion of approximately 18 Gm. of cinchophen over a period of eighteen days. This case showed evidence of the greatest amount of disturbance of hepatic function in the entire series (375 mg., or 78 per cent). Case 39 was that of a man with lobar pneumonia with toxic jaundice following the crisis. In this case the result of the test indicated definite damage of the liver (236 mg., or 49 per cent).

Case 41 is one of "cholangeitis lenta," diagnosed by biopsy, showing leukocytic infiltration of the periportal fields, especially around the bile ducts, thickening of the stroma and the retention of bile within the hepatic cells, with no evidence of cirrhosis. The excretion was 233 mg. (48.5 per cent), 218 mg. (45.5 per cent) and 226 mg. (47 per cent), respectively, on three tests made over a period of a week.

22. Klemperer, P.; Killian, N. A., and Heyd, C. G.: The Pathology of "Icterus Catarrhalis," Arch. Path. **2**:631 (Nov.) 1926.

TABLE 7.—*"Toxic Hepatitis" or Hepatosis; Chronic Hepatitis; "Cholangeitis Lenta"*

Case	Weight, Kg.	Temperature, F.	White Blood Cells	Jaundice	Icterus Index	van den Bergh Reaction		Retention of Bromsulphalein, per Cent in 30 Minutes	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
						Direct	Quantitative, Mg.				Mg.	Per Cent	
34	58.0	105.2	7,100	+	100	Prompt, positive	8.0	..	5/ 9	72	375	78.0	Toxic hepatosis (cinchophen)
	58.0	103.0	.....	+	150	.....	2.0	..	5/15	157	353	73.5	
	58.0	103.6	.....	+	100	.....	2.0	50	5/25	40	141	29.5	
35	67.5	99.0	.....	+	25	Negative	0.2	35	2/ 7	53	302	63.0	Hepatosis (hypertonic saline) infusion
	68.0	99.0	.....	Slight	..	.....	...	..	2/14	83	177	37.0	
36	37.0	100.2	11,800	+	75	Prompt, positive	3.4	5	4/ 3	0	125	26.0	Toxic hepatosis (phenobarbital); malnutrition
	35.0	99.0	.....	+	35	Negative	...	..	4/12	11	226	47.0	
	36.5	100.2	.....	0	15	.....	...	..	4/23	0	137	28.5	
37	62.0	98.6	.....	0	..	.....	...	..	2/14	12	169	37.5	Arsenical "hepatitis" (convalescent)
	60.0	98.6	.....	0	..	.....	...	..	2/23	25	117	25.0	
38	42.5	99.4	9,100	0	..	Prompt, positive	3.0	80	2/ 4	128	307	64.0	Chronic hepatitis; cholangeitis ?
	42.5	101.2	.....	0	30	.....	...	..	2/ 7	0	111	23.0	
	41.0	99.0	.....	0	..	.....	...	..	2/10	120	198	41.0	
	40.0	99.4	.....	0	25	Delayed, positive	1.0	55	2/16	23	158	33.0	
39	53.0	99.0	14,900	0 Previously	..	....	...	..	4/ 3	90	236	49.0	Toxic hepatitis (lobar pneumonia)
40	39.5	99.2	18,000	+	30	Negative	0.2	35	2/13	34	176	36.5	Chronic hepatitis
	39.5	99.4	.....	+	..	.....	...	..	2/17	2	191	40.0	
	38.5	99.8	.....	+	25	Negative	0.3	5	2/23	44	207	43.0	
	35.0	99.4	.....	+	18	.....	...	..	3/15	0	194	40.5	
41	57.0	99.6	11,300	+	70	Delayed, positive	2.6	..	7/ 1	63	233	48.5	"Cholangeitis lenta"
		99.8	.....	+	..	.....	...	..	7/ 3	50	218	45.5	
		99.8	.....	+	..	.....	...	..	7/ 5	23	226	47.0	

In this group of cases of diffuse disease of the liver, results above 200 mg. (42 per cent) were usually obtained. In the more severe cases with diffuse parenchymal degeneration, readings above 300 mg. (63 per cent) were observed.

CARCINOMA OF THE LIVER

One case of primary endocellular carcinoma of the liver and seven cases of metastatic carcinoma of the liver were studied (table 8).

The primary tumor of the liver diagnosed by biopsy occurred in a boy, aged 18 (case 42). The initial test showed an excretion of 295 mg. (61.5 per cent) with a corresponding retention of bromsulphalein of 50 per cent and a low icteric index of 20. During the succeeding three months, during which the patient received a course of radiotherapy, unfortunately he was not observed. The test now repeated showed a marked decline in the excretion of oxy-cinchophen to 108 mg. (22.5 per cent) or slightly above normal with a corresponding decrease in the retention of bromsulphalein to as low as 5 per cent in thirty minutes. The general condition of the patient remained unchanged, but the slight jaundice almost disappeared in the interval between the tests.

In the cases of metastatic carcinoma, regardless of their origin, the test gave results well below 200 mg. At necropsy in case 43, it was observed that the right lobe of the liver was replaced by a huge circular solid metastasis from a primary carcinoma of the hepatic flexure of the colon; the remaining part of liver appeared normal. The result in this case was 133 mg. (27.5 per cent).

It is noteworthy that tests repeated at short intervals may show a decline in the excretion of oxy-cinchophen, suggesting improved and even normal function. The cholerectic action of the test dose may possibly account for the apparent improvement in the function of the liver.

CARCINOMA OF THE PAPILLA OF VATER

Two cases of carcinoma of the papilla of Vater were studied (table 9). Case 48, confirmed by biopsy, was studied thirteen months following the resection of an adenocarcinoma of the papilla of Vater. Tests made before and after the establishment of drainage by secondary choledochostomy showed excretions of 294 mg. (61 per cent) and 265+mg. (55+per cent), respectively, the latter figure being incomplete, owing to leakage of bile through surgical dressings. The necropsy in this case showed degeneration of the liver.

In case 49 the diagnosis is not conclusive, as it rested on a palpatory laparotomy observation. With only slight disturbance of the function of the liver, an excretion of 144 mg. (30 per cent) and 122 mg. (25.5

TABLE 8.—*Carcinoma of the Liver*

Case	Weight, Kg.	Temperature, F.	White Blood Cells	Jaundice	Icterus Index	van den Bergh Reaction		Retention of Bromsulphalein, per Cent in 30 Minutes	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
						Direct	Quantitative, Mg.				Mg.	Per Cent	
42	49.0	100.2	10,200	+	20	Hemolyzed	0.2	50	3/28	41	295	61.5	Primary carcinoma of liver (biopsy); radiotherapy
	49.2	100.2	9,800	0	15	Prompt, positive	1.0	5	6/ 6	51	108	22.5	
43	....	99.4	.....	0	5	.....	...	10	2/20	78	133	27.5	Colloid carcinoma of hepatic flexure of colon; metastasis to liver (necropsy)
44	42.0	100.0	5,800	0	5	.....	...	..	3/25	15	154	32.0	Metastatic carcinoma of liver (biopsy)
	43.5	99.0	.....	0	..	.....	...	0	3/28	15	186	38.5	
45	48.0	99.2	.....	0	6	.....	...	15	2/26	30	63	13.0	Carcinoma of prostate; metastasis to liver
	47.5	99.4	.....	0	..	.....	...	..	3/ 8	22	149	31.0	
46	61.0	99.6	.....	0	..	.....	...	..	1/24	14	138	28.5	Carcinoma of liver; abdominal carcinomatosis; ascites
	61.0	99.0	.....	0	..	.....	...	..	1/28	0	98	20.5	
47	45.0	102.2	10,800	0	6	Negative	0.2	..	5/19	36	118	24.5	Carcinoma of stomach; metastasis to liver

TABLE 9.—*Carcinoma of Papilla of Vater and of Pancreas*

Case	Weight, Kg.	Temperature, F.	White Blood Cells	Jaundice	Icterus Index	van den Bergh Reaction		Retention of Bromsulphalein, per Cent in 30 Minutes	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
						Direct	Quantitative, Mg.				Mg.	Per Cent	
48	57.5	99.0	5,500	..	35	Negative	0.2	..	2/ 7	90	294	61.0	Adenocarcinoma of papilla of Vater resection; biliary fistula; cholangeitis; degeneration of the liver (necropsy)
	48.0	99.6	.....	0	16	.....	...	..	4/ 1	60	265	55.0	
49	58.0	98.6	6,200	+	150	Prompt, positive	7.0	..	5/21	60	144	30.0	Carcinoma of papilla of Vater (laparotomy) biliary fistula
	58.0	100.2	.....	+	..	.....	...	..	5/23	28	122	25.5	
	58.0	100.0	.....	+	..	.....	...	..	5/25	29	257	53.5	
	57.5	101.2	.....	+	..	.....	...	..	6/ 1	59	326	68.0	
	56.0	99.4	.....	+	..	.....	...	..	6/10	50	133	27.5	
50	....	100.8	.....	+	..	Prompt, positive	6.0	..	4/ 3	117	311	65.0	Carcinoma of head of pancreas (clinical)
51	....	99.8	8,100	+	..	Prompt, positive	7.0	..	2/18	50	219	45.5	Carcinoma of head of pancreas (clinical)
52	38.5	98.0	10,400	+	25	Delayed, positive	0.9	50	4/ 2	30	247	51.0	Carcinoma of body and tail of pancreas (necropsy)
53	....	100.4	16,400	+	125	Prompt, positive	3.0	..	4/22	135	367	76.5	Carcinoma of head of pancreas (clinical)
54	....	99.6	13,200	+	100	Prompt, positive	4.4	80	3/21	0	288	60.0	Carcinoma of head of pancreas (laparotomy)
		99.8	.....	+	..	.....	...	..	3/23	0	256	53.0	

per cent) at the outset, the excretion increased rapidly in the course of a week up to 326 mg. (68 per cent). Nine days after relief from the obstruction by choledochostomy performed under local anesthesia, the excretion was 133 mg. (27.5 per cent), the result being incomplete owing to leakage of bile through the dressings. The amount of oxy-cinchophen and cinchophen thus lost may have been considerable; it is difficult to estimate.

CARCINOMA OF THE PANCREAS

Five cases of carcinoma of the pancreas were studied (table 9). In one, the diagnosis was confirmed by necropsy (case 52), in a second by laparotomy (case 54) and in the remaining three, the diagnosis was clinical. In the first case the body and tail of the pancreas were involved, and the diagnosis was made before the appearance of jaundice, due to the presence of hyperglycemia, and roentgen evidence of chronic duodenal stasis caused by carcinomatous involvement of the duodeno-jejunal angle. Jaundice, which varied in intensity, was due to involvement of the lymph nodes of the porta hepatis and numerous small metastases in the liver. A single test revealed an excretion of 247 mg. (51 per cent) of oxy-cinchophen. The remaining four cases, 50, 51, 53 and 54 (case 54 was confirmed by laparotomy) were diagnosed as involving the head of the pancreas. Except for case 51, which is incomplete due to loss of some urine with the stool, the readings varied between 247 mg. (51 per cent) and 367 mg. (76.5 per cent).

The elevation in excretion of oxy-cinchophen above 200 mg. may be present even before the appearance of jaundice. The explanation of this degree of disturbance in hepatic function in carcinoma of the pancreas awaits further investigation. Whether the chronic partial obstruction of the common duct, the chemical changes in the duodenum due to the diminution or exclusion of pancreatic secretion, disturbance in the function of glycogen storage by the liver or actual pathologic changes in the liver, as have been observed in dogs following pancreatectomy,[23] play a rôle is at present a matter for conjecture.

Should these results be maintained in a larger series of cases, this test might aid in the differentiating jaundice due to stone in the common duct from that due to carcinoma of the pancreas.

HEPATIC DYSFUNCTION IN EXTRAHEPATIC DISEASE

This group comprises forty-seven cases (table 10); cases of duodenal ulcer, acute rheumatic fever, exophthalmic goiter, pulmonary disease,

23. Allan, F. N.; Bowie, J. D.; Macleod, J. J. R., and Robinson, W. L.: Behavior of Depancreatized Dogs Kept Alive with Insulin, Brit. J. Exper. Path. **5**:75, 1924.

TABLE 10.—*Extrahepatic Diseases with Increased Excretion of Oxy-Cinchophen*

Case	Weight, Kg.	Temperature, F.	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
					Mg.	Per Cent	
55	62.0	98.6	1/16	0	119	25.0	Duodenal ulcer
	63.0	99.0	2/ 3	0	102	21.0	
	60.0	99.4	2/15	38	153	32.0	
56	58.5	99.0	1/15	40	138	28.5	Duodenal ulcer; syphilis
57	58.0	99.0	5/ 1	32	110	23.0	Duodenal ulcer; syphilis
	58.0	99.0	5/ 3	18	102	21.0	
58	60.5	99.6	1/29	0	95	20.0	Acute rheumatic fever
	62.0	99.8	2/ 8	41	276	57.5	
	63.0	99.0	2/22	17	136	28.5	
59	72.0	99.2	1/27	74	167	35.0	Acute rheumatic fever (mitral and aortic disease)
	71.5	99.2	2/ 1	16	129	27.0	
	72.0	99.4	2/ 8	16	133	27.5	
60	59.0	98.6	2/25	38	181	37.5	Acute rheumatic fever (mitral and aortic disease)
61	55.0	99.4	1/14	63	120	26.0	Acute rheumatic fever
62	....	101.0	3/22	41	164	34.0	Lobar pneumonia; duodenal ulcer
63	57.0	98.6	1/20	0	212	44.0	Lobar pneumonia; duodenal ulcer
	57.0	98.6	1/25	5	119	25.0	
64	61.5	99.6	3/ 4	65	116	24.0	Lobar pneumonia; duodenal ulcer
	61.5	99.6	3/ 6	77	141	29.5	
65	41.0	99.0	4/16	0	104	21.5	Lobar pneumonia; duodenal ulcer
66	55.0	99.8	2/28	0	84	17.5	Bronchopneumonia; (interval of fever 100.4 F. prior to second test)
67	68.0	99.0	4/30	38	216	45.0	Bronchopneumonia
	68.5	99.0	5/ 2	0	153	32.0	
68	64.0	99.8	3/19	37	174	36.0	Bronchopneumonia; mucous colitis
	62.5	99.0	4/ 1	0	113	23.5	
69	88.0	99.8	1/28	19	162	34.0	Bronchopneumonia; bronchiectasis
	87.0	100.0	2/ 1	23	139	29.0	
	88.0	99.8	3/ 1	13	75	15.5	
	88.0	99.6	3/ 7	0	81	17.0	
70	70.5	99.2	2/20	39	120	25.0	Bronchiectasis
	70.5	99.0	6/ 1	51	150	31.0	
71	58.5	99.0	1/18	3	185	38.5	Pulmonary tuberculosis
	63.0	99.2	1/27	0	85	17.5	
	64.0	98.6	2/ 5	45	142	29.5	
72	58.5	98.6	2/19	95	305	63.5	Pleurisy with effusion
	58.5	98.6	2/25	0	74	15.5	Tuberculosis
73	52.0	99.4	1/30	30	155	32.5	Miliary tuberculosis (pulmonary); migraine
74	31.5	101.4	2/22	0	147	30.5	Tuberculosis of mediastinal lymph nodes
75	45.0	100.4	2/25	12	109	22.5	Neoplasm of left lung
76	65.0	99.2	2/27	30	119	25.0	Mitral stenosis and insufficiency
77	45.5	99.2	1/24	25	114	23.5	Chronic cardiovascular disease, mitral; aortic disease; auricular fibrillation; hydrothorax
78	63.0	99.0	1/17	38	107	22.5	Chronic cardiovascular disease, mitral; tricuspid disease; hydrothorax; ascites
79	55.9	99.0	1/21	40	117	24.5	Chronic cardiovascular disease; mitral; aortic disease; splenomegaly
80	60.0	103.8	1/15	0	153	32.0	Subacute bacterial endocarditis
	60.0	104.2	1/21	0	158	33.0	Aortic insufficiency
81	57.0	102.4	1/23	23	126	26.0	Subacute bacterial endocarditis; (healed) lupus erythematosis
82	65.0	99.0	2/14	75	162	33.5	Mitral stenosis and insufficiency
	65.0	100.8	2/22	64	176	36.5	Hepatosplenomegaly
83	44.0	99.2	2/ 8	18	134	28.0	Polycythemia vera
84	55.0	98.6	1/19	0	242	50.0	Polycythemia; splenomegaly
	55.0	98.6	2/17	36	131	27.5	
85	70.0	98.6	2/ 2	14	68	14.0	Polycythemia; hepatosplenomegaly
86	67.5	99.6	2/20	28	128	26.5	Hypertension; exophthalmic goiter
87	45.0	99.0	5/10	30	126	26.0	Hypertension
88	47.0	99.2	5/ 8	45	204	42.5	Exophthalmic goiter
89	87.5	98.4	2/14	8	146	30.5	Exophthalmic goiter
90	65.0	99.8	3/11	?	158	33.0	Left sciatic neuritis
91	61.0	99.4	2/26	8	174	36.0	Bronchial asthma
	60.0	98.8	3/ 8	32	111	23.0	
92	43.0	97.8	3/11	?	139	29.0	Subsiding tonsillitis
93	?	?	2/18	0	108	22.5	Multiple carcinomas of the bone (biopsy)

TABLE 10.—*Extrahepatic Diseases with Increased Excretion of Oxy-Cinchophen —Continued*

Case	Weight, Kg.	Temperature, F.	Date of Test	Excretion of Oxy-Cinchophen in Second 12 Hr., Mg.	Total Excretion of Oxy-Cinchophen in 24 Hours		Diagnosis
					Mg.	Per Cent	
94	52.0	99.4	2/18	0	128	26.5	Multiple carcinomas of the bone (biopsy)
95	48.0	102.0	2/20	45	150	31.0	Chronic lymphatic leukemia; Hodgkin's disease (necropsy)
96	50.0	100.4	4/30	53	150	31.0	Erythema multiforme
	53.5	99.4	5/ 2	105	178	37.0	
97	67.0	98.6	4/16	0	155	32.5	Pararectal disease; myocardial insufficiency
98	65.0	99.0	2/ 3	0	49	10.5	Penetrating gastric ulcer (roentgen examination)
	65.0	99.0	2/ 6	0	104	21.5	
99	57.0	101.0	1/28	46	110	23.0	Miliary carcinosis of lung
100	57.0	99.4	1/18	45	206	43.0	Ulcerocarcinoma of stomach; bronchopneumonia (necropsy)

chronic heart failure and a miscellaneous group are included. Cases of this type may show normal readings. In those with abnormal readings, the excretion of oxy-cinchophen is as a rule less than 200 mg. (42 per cent). Exceptionally, isolated readings as high as 300 mg. (63 per cent) occur in pneumonia, pleurisy with effusion and tuberculosis. The absence of frank jaundice in these cases indicates that moderate or even severe disturbance may occur in the metabolic functions of the liver without material disturbance in the excretion of bile.

COMMENT

A relatively constant excretion of oxy-cinchophen in the urine occurs with repeated test doses of cinchophen (0.45 Gm.) in the same normal subject. In patients with hepatic dysfunction, a higher excretion of oxy-cinchophen follows the ingestion of the test dose. Under uniform conditions in the same patient, this excretion is also constant. After a larger dose (0.9 Gm.), however, the patient with hepatic dysfunction excretes a variable, sometimes lower, sometimes higher, percentage of the dose as oxy-cinchophen.

If a small dose (0.1 Gm.) of oxy-cinchophen is fed to persons with normal and to those with abnormal function of the liver, only a fraction of it reappears in the urine, approximately the same amount as when an equal dose (0.1 Gm.) of cinchophen had been administered.

The clinical observations indicate a certain parallelism between the total excretion of oxy-cinchophen and the degree of damage to the liver. The following theoretical basis for these observations is suggested: The constant excretion of oxy-cinchophen following repeated test doses of 0.45 Gm., given orally, and also the larger dose (0.9 Gm.) of cinchophen in the same normal subject depends on a uniform rate of absorption and a constant metabolic rate for cinchophen in the liver. With these doses a relatively constant amount of cinchophen is con-

verted to oxy-cinchophen and the remainder disposed of in some other manner (excreted as cinchophen, oxypyridinuric acid,[19] etc.). With a larger dose of cinchophen (0.9 Gm.), the abnormal liver cannot maintain this constant metabolic rate, larger fractions of the ingested dose fail to be metabolized beyond the stage of oxy-cinchophen, and therefore larger amounts of this oxidation product appear in the urine.

Thus oxy-cinchophen probably represents an intermediary product in the destruction of cinchophen by the liver. The abnormal cells of the liver convert cinchophen to oxy-cinchophen, but the subsequent catabolism of this substance proceeds at a slower rate and to a lesser degree. With increasing disturbance of the function of the cells of the liver, larger and larger fractions of the standard dose are consequently eliminated in the urine as oxy-cinchophen.

It has been contended that because of the manifold functions of the liver and the great reserve of liver tissue, a test of any single function cannot be used as an index of function of the organ as a whole. My test, however, depends on a metabolic function of the liver that apparently is among the first to be disturbed and moreover is apparently disturbed in proportion to the extent and severity of the disease. Therefore, I believe that it offers the possibility of a quantitative index of the extent of damage or dysfunction of the cells of the liver.

The present test is not to be considered a tolerance test for cinchophen, since the excretion of one of its metabolic conversion products, and not of the unaltered substance, is measured.

The interpretation of the results of the test is based on a series of approximately fifty cases of disease of the liver and the gallbladder and fifty of nonhepatic disease in which evidence of hepatic dysfunction was obtained. The diagnostic possibilities of the test as indicated in the discussion of the different groups of cases must be further applied in a larger series of carefully studied cases to determine its clinical value.

SUMMARY

A practical, simple and extremely sensitive colorimetric method for the estimation of 2-(ortho-hydroxy-phenyl)-quinoline-4-carboxylic acid (oxy-cinchophen) is described, based on the characteristic Skorczewski-Sohn color reaction and revealing this substance in dilutions of 0.0002 per cent.

This test for the function of the liver is based on the estimation of the twenty-four hour excretion of oxy-cinchophen in the urine following the ingestion of a standard dose of 0.45 Gm. of cinchophen. In a group of fifty subjects without hepatic dysfunction, from 30 to 100

mg., or from 7 to 21 per cent, of the test dose, was excreted as oxy-cinchophen.

The increase in excretion of oxy-cinchophen in the urine may be useful as a quantitative index of an altered metabolism of the cells of the liver whereby they have lost their capacity to decompose this substance further.

Biliary stasis or obstruction per se does not materially influence the results of the test, at least not early in the disease.

The ingestion of a standard test dose of 0.45 Gm. of cinchophen has not elicited any hepatotoxic effects.

A limited clinical experience with the test suggests certain diagnostic opportunities. The results indicate that in biliary obstruction due to stone or stricture and in metastatic carcinoma of the liver the daily excretion of oxy-cinchophen is usually less than 200 mg. (42 per cent), while in diffuse degenerative and acute inflammatory lesions involving the parenchyma of the liver more than 200 mg. is excreted in twenty-four hours. In cirrhosis of the liver the results may be normal or below 200 mg. in the chronic stage and above 200 mg. only when an acute or subacute degeneration of the liver is superimposed. In catarrhal jaundice the results are usually below 200 mg. with an occasional sharp increase on repeated tests. In carcinoma of the pancreas a persistent increase above 200 mg. has thus far been obtained.

In extrahepatic diseases, such as chronic heart failure, duodenal ulcer, exophthalmic goiter, acute rheumatic fever and pneumonia, there is sometimes an increase in the excretion of oxy-cinchophen.

PHYSOSTIGMINE SALICYLATE IN THE TREATMENT OF EXOPHTHALMIC GOITER

OBSERVATIONS ON TWO HUNDRED CASES*

ISRAEL BRAM, M.D.
PHILADELPHIA

It can safely be assumed that irrespective of the form of treatment advocated or adopted for sufferers from exophthalmic goiter, the uppermost immediate aim of the physician is a reduction of the heart rate to normal. Many clinical facts justify this aim. The heart bears the brunt of the damage wrought by the intensity and duration of exophthalmic goiter, and cardiac failure is the usual cause of death. The height of the heart rate and the output of blood usually parallel the severity of the other manifestations of the disease; likewise, amelioration of symptoms or recovery of the patient is announced by a calming of the heart rate and force. Indeed, so constantly is a significant behavior of the heart associated with the varying phases of this syndrome that the heart's action may be regarded as a most reliable criterion of the course of the disease during crisis, remission or recovery as the case may be.[1]

DIGITALIS IN TREATMENT

The tachycardia of exophthalmic goiter is characterized by features that in combination assist considerably in its diagnosis. These are (*a*) its afebrile character; (*b*) its association with a high basal metabolic rate; (*c*) its chronicity, being weeks, months or years in duration; (*d*) its constancy, being but little altered by rest or sleep, and (*e*) its resistance to the influence of digitalis even in large doses.

Personal (unpublished) observations conducted on a series of 100 cases ten years ago revealed that digitalis may be of benefit in exophthalmic goiter in approximately 50 per cent of the cases only under the following conditions: (1) in combination with quinidine sulphate in efforts at restoration of rhythm in a fibrillating heart; (2) during approaching or actual remission; (3) during impending or actual circulatory decompensation, and (4) when in the favorable course of the disease improvement in the condition of the heart lags behind obvious

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1. This exception must be noted: In spontaneous or induced myxedema complicating or modifying the disease, the heart rate may be normal or below without recovery of the patient, since circulatory calm may mask a residual or potentially active Graves' syndrome.

improvement elsewhere; in other words, when under successful treatment, the basal metabolic rate, weight, sense of well-being and other clinical evidences point to impending recovery, but the heart rate, probably from force of habit of the tissues, is still 80 or over, the administration of digitalis may succeed in its purpose. In those observations we sought to evaluate the effects of digitalis on the tachycardia of active, previously untreated cases of exophthalmic goiter and found the drug practically valueless as far as the heart rate was concerned. In 10 of the series of 100 patients the dose of the standard tincture of digitalis was increased to 1 drachm three times a day for a period of three weeks with no discernible lowering of the heart rate. Indeed, in 3 of these patients, there resulted an increase in the heart's excitability. In 5 of the 10 patients, the dosage was increased to 2 drachms three times a day for an added week. This aggravated the entire syndrome. It appears that the drug created a sort of vicious circle, namely, it engendered digestive disturbances and an aggravation of the syndrome, and this in turn increased the heart's rate.

These comments are applicable also to the various derivatives of digitalis and to the commonly employed "heart stimulants," including strophanthus, convallaria root and sparteine. Our observations of a decade ago included the use of practically all of the popular drugs for the action of the heart, and the conclusion drawn was that as far as the tachycardia of active exophthalmic goiter was concerned, the use of these substances was without tangible benefit.

It is evident that success in the treatment of tachycardia in exophthalmic goiter may be expected only when measures are directed toward the cause of cardiac excitability. Whether this rests primarily in an excess of thyroid hormone in the blood is still a moot question related to the controversial problem of whether exophthalmic goiter and toxic adenoma are different manifestations of the same disease. With regard to the direct or immediate cause of excessive cardiac activity, the consensus points to an overactivity of the cardiac accelerator nerves issuing from the sympathetic nervous system. The tachycardia appears to be the result of a dominating sympatheticotonia, over which the checking influence of the vagus nerve is insufficient to maintain a normal heart rate.

It is, therefore, reasonable to assume that any measure capable of alleviating sympatheticotonia either directly or by stimulation of the vagus nerve or both could rightly claim an important place in the treatment of exophthalmic goiter. Also it appears reasonable to infer that such a measure could favorably influence other sympatheticotonic evidences as exophthalmos and the intestinal sluggishness observed in a percentage of cases.

PHYSOSTIGMINE SALICYLATE VERSUS SYMPATHETICOTONIA

Our observations during a period of one year (ending June, 1930) in a series of 200 cases of active exophthalmic goiter indicate that physostigmine is a remedy largely conforming with the requirements mentioned. While the literature on the subject is rather meager, yet occasional mention is made of the benefit derived from the administration of this drug in cases of tachycardia with and without involvement of the thyroid. Among the contributors are Mougeot,[2] Lian and Welti,[3] Moutier,[4] de Meyer,[5] and Plitman and Ender.[6]

According to Cohen and Githens,[7] physostigmine exerts its action chiefly on the autonomic nervous system, slowing the heart by a stimulating influence on the cardiac endings of the vagus nerve. There is also evidence to indicate that physostigmine exerts a depressing effect on the cardiac accelerator fibers issuing from the sympathetic nervous system. In our series of observations, physostigmine reduced the force and rate of the heart's action in approximately 73 per cent of the cases, with resultant improvement in subjective and objective symptoms. Moreover, this drug exerts a beneficial influence on exophthalmos and overcomes defective intestinal elimination. So valuable a substance did we find physostigmine to be that it is felt that its use should become widespread as an important constituent in the armamentarium of all clinicians who have patients of this sort under their care. Theoretically, it would appear that a reduction of heart rate and force would reduce the vascularity and size of the thyroid gland and consequently the excitability of this organ. This actually occurred in quite a few cases in this series.

CLINICAL FEATURES IN THE TWO HUNDRED CASES OF EXOPHTHALMIC GOITER IN WHICH PHYSOSTIGMINE SALICYLATE WAS EMPLOYED

The age of the patients ranged from 7 to 64 years, with an average of 31 years. There were 142 females, or 71 per cent, and 58 males, or 29 per cent. The duration of the illness, as stated by the patients

2. Mougeot, J.: Physostigmine Test in Cardiology, Bull. et mém. Soc. méd. d. hôp. de Paris **45**:512, 1921.

3. Lian, C., and Welti, H.: Treatment of Tachycardia, Bull. et mém. Soc. méd. d. hôp. de Paris **45**:559, 1921.

4. Moutier, F.: Eserine in Internal Therapeutics, Paris méd. **11**:453, 1921.

5. de Meyer, J.: Intermittent Acceleration of Sinus Rhythm, Arch. d. mal. du coeur **15**:265, 1922.

6. Plitman, M., and Ender, S.: Reinforcement of Physostigmine by Serum Through Action of Thyroid, Ztschr. f. d. ges. exper. Med. **57**:361, 1927.

7. Cohen, S. S., and Githens, T. S.: Pharmacotherapeutics, New York, D. Appleton & Company, 1928, p. 1780 and 1782.

when first observed, varied from four weeks to twenty years, with an average of fifteen months. The basal metabolic rate ranged from +14 to +82 per cent, the average being +46 per cent. The heart rate varied in different patients from 82 to 180 a minute, with an average of 106. Arrhythmia and auricular fibrillation were present in 36 cases, or 18 per cent. Exophthalmos in varying degree was present in 134 cases, or 67 per cent.

The treatment employed by the patients prior to coming under our attention varied. Fourteen, or 7 per cent, had had thyroidectomy performed with little or no relief; 26, or 13.2 per cent, had undergone roentgen or radium therapy without marked benefit; 116, or 58 per cent, had received various forms of medical attention, particularly the administration of iodine and rest cures; and 44, or 22 per cent, had received no previous attention whatever.

It may be added that in the selection of these cases reasonable care was exercised to exclude patients presenting a dominating vagotonic symptomatology with a relatively slow heart rate. The patients selected for the use of physostigmine were dominantly of the sympatheticotonic type.

DOSAGE AND CONTRAINDICATIONS

The dosage of physostigmine employed varied between 1/60 and 1/30 grain (1 and 2 mg.) three times a day, depending on the weight of the patient. In the majority of instances the dosage of 1/30 grain three times a day was employed in patients weighing approximately 120 pounds (54.4 Kg.) or more. In those weighing less and in children, the dosage was correspondingly smaller. While it is recognized that this dosage of physostigmine salicylate is considerably in excess of the arbitrary dose, it was discovered in this series that smaller doses were scarcely capable of influencing the patient's symptomatology, and that the dosage employed was almost invariably well tolerated.

Physostigmine was administered in each case for a period of ten weeks. At the termination of this period most patients had received the maximum benefit from the drug, and it was discontinued. In those who did not show benefit from its use within three or four weeks, the drug was withdrawn.

While theoretically such symptoms as diarrhea and sweating would seem to deter the administration of physostigmine, such was rarely the case in this series. In only five cases was it necessary to discontinue the drug because of diarrhea. Indeed, in the majority of patients presenting diarrhea as a marked symptom physostigmine, by virtue of its potentiality for general improvement in circulatory and sequentially in nervous excitability, improved the intestinal function. Here we must not overlook the cases of apparent intestinal excitability resulting from

retention of or irritation by intestinal contents. In such patients, diarrhea was improved because of the better elimination of intestinal residue. In no instance was it necessary to withhold or withdraw physostigmine because of excessive sweating. It appears that the circulatory stabilization following the administration of physostigmine is largely the corrective of cutaneous symptoms of exophthalmic goiter.

COURSE OF THE DISEASE UNDER TREATMENT WITH PHYSOSTIGMINE

On the firm conviction that no single measure is capable of effecting satisfactory relief from exophthalmic goiter, these patients were also subjected to such additional measures as a high caloric diet of minimal animal protein content; a rest cure involving a stay in bed of from ten to fourteen hours (depending on the severity of the case); the administration of quinine or quinidine and an occasional medicament to overcome insomnia, digestive disturbances and other symptoms from time to time requiring attention; the correction of infectious foci wherever possible, and finally, the application of practical psychotherapy which in occasional instances extended to relatives or friends interested in the patient's welfare.

With a view to checking up the benefit assignable to the administration of physostigmine alone, the records of another 100 cases of exophthalmic goiter in which no physostigmine was given were studied as controls. These patients were otherwise managed in exactly the same manner as were those to whom physostigmine was administered. It was found that in patients receiving the drug the results obtained, particularly those referable to the heart, occurred several weeks before tangible improvement in patients not receiving physostigmine, and the tendency toward the cyclic flaring up of symptoms or crises (either spontaneous or induced by psychic trauma) was decidedly less frequent.

Primarily the object of the administration of physostigmine was the lowering of the heart rate. Not alone was this accomplished in 146 cases, or 73 per cent, of this series, but in the majority, the force of the heart's action was reduced to the extent of the total elimination of subjective complaints referable to the precordium. Commonly the patient would remark that the thumping of the heart against the ribs and the consciousness of the heart's action had disappeared.

In patients suffering with auricular fibrillation, physostigmine alone seemed more efficacious than quinidine alone, but the combination of physostigmine and quinidine was very efficacious in the control of cardiac excitability and the restoration of rhythm. The combination of physostigmine salicylate, 1/30 grain, and quinidine sulphate, 5 grains (0.324 Gm.), three times a day, was employed.

Irrespective of the form of treatment adopted in exophthalmic goiter, improvement in exophthalmos is generally observed to be tardy. Even under expert thyroidectomy not only does exophthalmos persist in a large percentage of cases, but in some patients with exophthalmic goiter without exophthalmos this symptom develops postoperatively.[8] At best, improvement in exophthalmos is among the last events in the clearing up of the syndrome, and in many patients the eyes never become normal. A measure to improve exophthalmos in a more or less specific fashion has been the crying need through the years. In view of the results observed in this series of patients, it is felt that the administration of physostigmine is the closest approach to such a measure. The results were gratifying in many instances. The eyes of patients under treatment with physostigmine are apt to improve simultaneously with manifest improvement elsewhere, and a good percentage of patients with and without a history of thyroidectomy suffering with residual exophthalmos of years' duration has been greatly benefited or completely relieved of this distressing symptom.

VARIABLES IN RESULTS OF THE ADMINISTRATION OF PHYSOSTIGMINE

The patients who were benefited to the greatest degree by the administration of physostigmine may be classified under six headings: (1) the extremes of age, i. e., patients under 14 and above 50; (2) those approaching remission after having had one or more crises; (3) sufferers from this disease over a period of four or more years, i. e., the chronic or protracted forms of exophthalmic goiter; (4) those presenting a basal metabolic rate of less than +40 per cent; (5) those presenting extreme exophthalmos, and (6) those presenting cardiac arrhythmia, especially auricular fibrillation.

On the other hand, patients who were least benefited by the administration of physostigmine were young adults presenting a basal metabolic rate of +40 per cent or higher and those who were obviously in crisis associated with rather marked cerebral excitation. However, the use of this drug was generally productive of good results when the crisis was passed.

END-RESULTS

Since statistical records of subjects with exophthalmic goiter would of necessity require the personal following up of those dubbed "cured" or "recovered" for a minimum of from three to five years, it is not in the scope of this paper to discuss the matter of end-results in this series of patients. This series has not been observed for a time of

8. Zimmerman, L. M.: Exophthalmos Following Operations for the Relief of Hyperthyroidism, Am. J. M. Sc. **178**:92, 1929.

sufficient length to permit of statements pertaining to end-results. In 98 patients, or 49 per cent, the pulse rate has remained normal and there is a maintenance of the general improvement to date. From present indications it appears likely that at the termination of a follow-up period of sufficient duration, the statistical records will compare favorably with those proceeding from other modes of treatment in the management of these patients. This will be the subject of another contribution in the course of time.

SUMMARY AND CONCLUSIONS

1. The results of the administration of physostigmine for a period of ten weeks in 200 cases of active exophthalmic goiter are discussed.

2. In ninety-eight, or 49 per cent, the benefit obtained was highly encouraging and may be lasting; in forty-eight, or 24 per cent, the benefit was transient though tangible, and in the remaining fifty-four, or 27 per cent, no benefit was discerned.

3. Excepting in five patients suffering from persistent diarrhea, no contraindications or untoward results were noted from the administration of physostigmine salicylate in doses corresponding to 1/30 grain three times a day in adults.

4. The most satisfactory results were noted: (1) in the extremes of age, i. e., in patients under 14 and above 50; (2) in those approaching remission after having had one or more crises; (3) in sufferers from this disease over a period of four or more years, i. e., the chronic or protracted forms of exophthalmic goiter; (4) in those presenting a basal metabolic rate of less than +40 per cent; (5) in those presenting extreme exophthalmos, and (6) in those presenting cardiac arrhythmia, especially auricular fibrillation.

5. The improvement in the behavior of the heart was the first effect noted. A reduction in rate and force of the heart's action was followed by improvement in the basal metabolic rate and all subjective and objective manifestations of the disease.

6. Improvement in exophthalmos in many instances of chronic and stubborn cases was very gratifying. In a few cases of postoperative recurrence with excessive exophthalmos, physostigmine was particularly satisfactory in results.

7. From the observations herein noted it appears that physostigmine deserves high regard in the management of exophthalmic goiter.

1633 Spruce Street.

STAINING OF RETICULOCYTES BY BRILLIANT CRESYL BLUE

INFLUENCE OF SOLUTIONS OF SUBSTANCES *

CLARK W. HEATH, M.D.
AND
GENEVA A. DALAND, S.B.
BOSTON

In recent years it has become more and more certain that the increase in the circulation of young red blood cells, which contain reticular substance when supravitally stained, is indicative of increased erythropoiesis. Clinical proof of this has been obtained by innumerable examples of the production of reticulocyte crises in response to various therapeutic procedures, and notably in cases of pernicious anemia following the institution of liver therapy, first described by Minot and Murphy.[1] As the importance of these young cells becomes more evident, the nature and behavior of the reticular or basophilic substance contained within them are arousing an increasing amount of interest. This basophilic substance in erythrocytes, closely related to the maturation of these cells, may well be a type of substance present in many other cells, and indeed may prove to be important in the elucidation of many clinical problems, notably those concerned with neoplastic diseases. The red blood cell, because of its clear cytoplasm, lends itself to ease of study of visible inclusions, the observation of which in other cells is hindered by their more complicated structure.

Although much remains to be learned about the basophilic substance present in reticulocytes, certain definite information has been obtained by various investigators. These data have been reviewed briefly in another paper,[2] in which we discussed the maturation of reticulocytes. It is a generally accepted fact that diffuse basophilia and reticular substance are identical, the latter being a manifestation of the former, pro-

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* From the Thorndike Memorial Laboratory, Boston City Hospital and the Department of Medicine, Harvard Medical School.

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1. Minot, G. R., and Murphy, W. P.: Treatment of Pernicious Anemia by a Special Diet, J. A. M. A. **87**:470 (Aug. 14) 1926.

2. Heath, C. W., and Daland, G. A.: The Life of Reticulocytes: Experiments on Their Maturation, Arch. Int. Med. **46**:533 (Sept.) 1930.

duced by the supravital action of certain dyes (Hawes,[3] Brückner and Spatz,[4] Schilling-Torgau,[5] Schilling,[6] Key [7] and Gawrilow [8]). The fact that the basophilic substance has different appearances for different conditions of staining has attracted much attention. Brückner [9] pointed out that the drying of red blood cells at elevated temperatures (from 60 to 100 C.) produces relatively more polychromatophilia than granulation, whereas lower temperatures produce more granulation than polychromatophilia. He stated that the relative number of polychromatophilic and granular cells is a function of the temperature and the moisture of the air in which they have been dried. The presence of diffuse basophilia and the absence of reticulation in ordinary Romanowsky stained preparations (Wright's stain) are explained by the action of strong fixing agents, such as methyl alcohol. Sudden fixing, as with osmic acid vapor, and subsequent staining produce the appearance of diffuse basophilia. Key [7] stated that a picture resembling punctate basophilia could be obtained by heating reticulated cells to 50 C. for one-half hour or by treating them with dilute potassium hydroxide. Brookfield,[10] by using weaker solutions of brilliant cresyl blue, found that reticulation became fragmented and resembled stippling, and that diffuse basophilia was present under such conditions. Schilling [6] described the net structure, or reticular substance, as a coarse, precipitated form of the diffusely spread material, giving the appearance termed polychromasia. Hawes [3] made counts of the basophilic cells and reticulocytes of the blood of various patients and found the percentage of basophilic cells always slightly lower than the percentage of reticulated cells. He explained

3. Hawes, J. B.: A Study of the Reticulated Red Blood Corpuscle by Means of Vital Staining Methods: Its Relation to Polychromatophilia and Stippling, Boston M. & S. J. **161**:493, 1909.

4. Brückner, H., and Spatz, R.: Ueber die Beurteilung des roten Blutbildes bei der Bleivergiftung unter Berücksichtigung verschiedener Darstellungsmethoden der polychromaten und basophil punktierten Erythrocyten, Arch. f. Hyg. **97**:277, 1926.

5. Schilling-Torgau, V.: Arbeiten über die Erythrozyten: I. Ueber die polychromophilic und verwandte Zustande, Folia haemat. **11**:327, 1911.

6. Schilling, V.: Die Zelltheorie des Erythrocyten als Grundlage der klinischen Wertung anämischer Blutbefunde, Virchows Arch. f. path. Anat. **234**:548, 1921.

7. Key, J. A.: Studies on Erythrocytes, with Special Reference to Reticulum, Polychromatophilia and Mitochondria, Arch. Int. Med. **28**:511 (Nov.) 1921.

8. Gawrilow, R.: Zur Lehre über die vitalfärbbare Substanz der Erythrozyten, Folia haemat. **38**:216, 1929.

9. Brückner, H.: Arbeiten über die basophile Substanz in den jugendlichen roten Blutkörperchen: II. Ueber die physikalisch-chemischen Eigenschaften der basophilen Substanz in den jugendlichen Erythrocyten, Arch. f. Hyg. **98**:95, 1927.

10. Brookfield, R. W.: Blood Changes Occurring During the Course of Treatment of Malignant Disease by Lead, with Special Reference to Punctate Basophilia and the Platelets, J. Path. & Bact. **31**:277, 1928.

this by the fact that it is difficult to distinguish a cell with a small amount of basophilia from a nonbasophilic cell, while it is easy to distinguish a reticulated cell with a small amount of reticular material from a nonreticulated cell. The two counts were parallel, and Hawes considered the two types different manifestations of the same substance.

When liver therapy was first being employed in cases of pernicious anemia, the mechanism of the action of the active principle in liver on the production of red blood cells stimulated Dr. George R. Minot to investigate the effect of liver extracts on reticulocytes. It was recognized that an in vitro method of determining the potency of liver extracts would be of great value. Efforts to obtain such a method met with no success, but the peculiar influence of extracts of liver and other substances on the staining of reticulocytes seemed to warrant special study.[11]

METHODS

Since Cesaris-Demel [12] published accounts of the supravital action of brilliant cresyl blue on reticulocytes, this dye has been widely used in the study of these cells. A solution of 0.5 per cent brilliant cresyl blue (National Aniline Company) in 95 per cent ethyl alcohol was used in the experiments to be described.

Blood was obtained from patients and from rabbits. Reticulocytosis was produced in the rabbits by the intraperitoneal injection of phenylhydrazine or by bleeding. The blood of patients with pernicious anemia at the height of the reticulocyte rise, following liver therapy, served as the source for human reticulocytes. The blood was collected under sterile precautions, placed in a sterile flask containing glass beads and defibrinated by gentle shaking. Sometimes, if the number of reticulocytes was low, the blood was centrifugated to concentrate the young cells at the top of the column of blood.

The substances to be tested were dissolved in distilled water, in physiologic solution of sodium chloride or in a buffer solution, composed of 0.12 molar of disodium phosphate and 0.03 molar of monosodium phosphate. Certain solutions of amino-acids and liver extracts were neutralized with sodium hydroxide, bromthymol blue being used as an indicator. As a rule, 0.4 cc. of solution was mixed with 0.2 cc. of blood in a small test tube. After the cells and solution had been in contact for a certain period of time, the test tube was shaken thoroughly and a small drop smeared between two cover slips on which brilliant cresyl blue had previously been dried. The cresyl blue smears were dried in the air and counterstained with Wright's stain (a modification of the Romanowsky stain, in which fixation is produced by methyl alcohol) after the methods of Hawes [3] and Cunningham.[13] Control preparations of the original blood were made in a similar manner. Permanent preparations mounted in balsam were studied for the relative numbers of cells showing diffuse basophilia and those showing reticular substance.

11. Drs. George R. Minot, Franklin R. Miller, Samuel S. Ellis and J. Seabury Hathaway of this laboratory did the pioneer work on this subject.

12. Cesaris-Demel, A.: Studien über die roten Blutkörperchen mit den Methoden der Färbung in frischen Zustande, Folia haemat. **4**:1, 1907.

13. Cunningham, T. D.: A Method for Permanent Staining of Reticulated Red Cells, Arch. Int. Med. **26**:405 (Oct.) 1920.

One thousand cells were counted, and the percentage of reticulocytes determined. In some cases the percentage of basophilic cells was also determined.

Some experiments were performed with cells washed free from serum with physiologic solution of sodium chloride. No marked difference was observed between the staining character of cells left in their own serum and cells washed free from serum, but the latter tended to hemolyze more easily than the former. A more pronounced effect of a substance could sometimes be demonstrated by the use of 0.6 cc. or 0.8 cc. of solution and 0.2 cc. of blood.

In the first series of experiments, 10 per cent solutions of the substances were usually used to test their effect on the staining of reticulocytes by brilliant cresyl blue. If this concentration was too toxic to red cells, as shown by hemolysis of the cells in preliminary test tube experiments or by the disintegration of the cells in the process of fixing and staining, such weaker strengths of the solutions were employed as did not produce these changes in the red blood cells.

THE INFLUENCE OF SOLUTIONS OF DIFFERENT SUBSTANCES

A summary of the results of experiments in which a variety of organic and inorganic substances was used is recorded in table 1. After the blood had remained in contact with a solution for about fifteen minutes and the smears had been stained, the percentage of reticulocytes, in most instances, diminished markedly, or the reticulocytes apparently entirely disappeared. A study of the preparations, however, revealed the presence of many diffusely basophilic cells, the number of which varied with the number of reticulocytes seen in the control preparations. Examples of such observations are shown in table 2. When only a few reticulocytes remained, diffusely basophilic cells were also seen. Cells which were diffusely basophilic in such preparations frequently contained strands of reticular substance or fine granules of basophilic staining substance. Cells could be found which varied greatly in the relative amounts of diffuse basophilia and reticular substance which they contained.

A wide variety of substances was chosen in order to determine whether or not this effect, antagonistic to the staining of reticular substance, was related to any one class of substances. It is evident from the data in table 1 that there was no such relationship. Organic and inorganic substances, strong and weak electrolytes and acids and bases produced this effect.

The substances most thoroughly studied were liver extracts.[14] Two typical examples of their effect on the staining of reticular substance are shown in table 1. Studies were made on liver extracts which were potent but which were impotent when given to patients with pernicious anemia. The influence of these extracts on the staining of reticulocytes bore no relation to their potency.

14. Many of these were prepared by Dr. Edwin J. Cohn and his associates (Cohn, E. J.; Minot, G. R.; Alles, G. A., and Salter, W. T.: The Nature of the Material in Liver Effective in Pernicious Anemia, J. Biol. Chem. **77**:325, 1928).

TABLE 1.—*The Influence of Solutions of Substances on the Staining of Reticulocytes with Brilliant Cresyl Blue*

Substance	Concentration of Solution, per Cent	Amount of Solution, Cc.	Amount of Defibrinated Blood, Cc.	Reticulocytes	
				Control, per Cent	After 15 Min. Exposure to Solution, per Cent
Liver extract no. 343 (N.N.R.)	15.0	0.4	0.2	29.3	5.8
Purified extract RI XLII*	10.0	0.4	0.2	12.2	0.6
Glycine	10.0	0.4	0.2	36.7	3.0
Alanine	10.0	0.4	0.2	36.7	10.2
Leucine	10.0	0.4	0.2	36.7	0.6
Glutaminic acid	1.0 (saturated)	0.4	0.2	20.0	10.6
Cystine	Saturated	0.4	0.2	27.1	24.4
Aspartic acid	Saturated	0.4	0.2	27.1	1.0
Sodium asparaginate	10.0	0.4	0.2	27.1	4.1
Creatinine	8.7	0.4	0.2	36.7	3.6
Dextrose	8.0	0.8	0.2	34.3	2.2
Glycocholic acid	±0.3	0.4	0.2	36.7	32.0
Phenylhydrazine	2.0	0.4	0.2	36.7	35.6
Lecithin	Small particle (insoluble)	...	0.2	27.1	29.0
Cholesterin	Small crystal (insoluble)	...	0.2	27.1	±30.0†
Peptone	10.0	0.4	0.2	26.6	17.2
Sodium chloride	2.5	0.6	0.2	20.4	0.4‡
Sodium chloride	2.5	0.4	0.2	21.2	3.4
Sodium iodide	2.5	0.4	0.2	19.8	0.0
Sodium sulphate	10.0	0.4	0.2	30.1	4.0
Disodium acid phosphate	5.0	0.8	0.2	19.8	11.2
Calcium chloride	7.5	0.4	0.2	26.6	0.0
Magnesium sulphate	10.0	0.4	0.2	30.1	0.0
Sodium hydroxide	0.001N	0.4	0.2	15.8	9.3
Hydrochloric acid	0.05N	0.4	0.2	15.8	10.6
Ammonium hydroxide	1.0	0.4	0.2	26.6	19.4
Acetic acid	0.1	0.4	0.2	26.6	5.2
Lactic acid	0.1	0.4	0.2	15.8	16.4
Ammonium acetate	10.0	0.4	0.2	26.6	19.4
Hydrogen peroxide	5.0	0.4	0.2	26.6	26.6
Glutathione	5.0	0.1	0.4	18.8	0.0
Pituitary extract (Lederle)	...	0.4	0.2	12.2	0.0
Epinephrine chloride 1:1,000	...	0.4	0.2	15.8	1.6
Thyroxin	Crystals	...	0.2	12.2	12.0

* Prepared by Dr. Edwin J. Cohn.
† Considerable injury to the red blood cells prevented accurate counting.
‡ Five minute exposure.

TABLE 2.—*Presence of Basophilic Cells and Reticulocytes After Contact with Different Solutions*

Substance	Concentration of Solution	After 15 Min. Contact With Solution Relative Number of Cells Showing	
		Reticular Material, per Cent	Diffuse Basophilia, per Cent
Control	Defibrinated blood	21.4	0
Control	Buffer solution and defibrinated blood	17.6	0
Liver extract no. 343 (N.N.R.)	20%	9.6	2.4
	20%	11.1	4.8
Glycine	10%	1.6	6.0
	10%	1.8	6.0

As these liver extracts were known to be rich in amino-acids, the latter alone were next studied. Glycine, alanine and leucine inhibited the staining of reticular substance by brilliant cresyl blue in a manner similar to that of corresponding strengths of liver extract no. 343 (N.N.R.). Cystine, in a dilute solution, had only a slight inhibiting effect. It is noteworthy that the two dibasic amino-acids, glutaminic and aspartic acids, had as strong an inhibiting influence as the monobasic acids, although their slight solubility allowed the use of only weak solutions. Dextrose behaved similarly to liver extracts and the amino-acids. In some qualitative experiments sucrose also had an inhibiting influence.

Sodium salts, calcium chloride and magnesium sulphate inhibited the staining of reticulocytes, the last two being apparently more effective than the sodium salts. The presence of a bivalent cation may account for this difference. Sodium hydroxide, hydrochloric acid, ammonium hydroxide and acetic acid, although they could be employed only in very small concentrations, seemed to have some inhibiting influence. Lactic acid, in one experiment, had no influence. Hydrogen peroxide itself was not effective. When added to a solution of liver extract or glycine, hydrogen peroxide did not alter the inhibiting effect of these substances on the staining of reticulocytes.

Extracts of pituitary and epinephrine were tested to compare their action with that of liver extracts, and they were found to have a similar inhibiting influence. Insulin, the action of which on the permeability of erythrocytes has been the subject of many reports,[15] likewise seemed to be effective. However, as insulin was toxic to the red blood cells in the strength employed, it became impossible to count the reticulocytes accurately.

The presence of diffuse basophilia in these preparations was as much a criterion in the estimation of the influence of a substance on the staining of reticulocytes as the actual reduction in the number of cells showing reticular material. When the influence of substances insoluble in water, such as cholesterin, lecithin and thyroxin, was studied, no diffuse basophilia could be seen, and there was no reduction in the number of reticulocytes. Therefore, these substances when added to blood were noneffective.

There is necessarily a limitation of the number of substances that can be tested in this manner, because of injury to the red blood cells which is caused when strong concentrations are used, whereas weak concentrations will not affect the cresyl blue staining. Such, for example, are urea, malonic acid, trivalent salts such as ferric chloride and

15. Haldane, J. B. S.; Kay, H. D., and Smith, W.: The Effect of Insulin on Blood Volume, J. Physiol. **59**:193, 1924. Irving, J. T., and Kay, H. D.: The in Vivo Permeability of the Red Corpuscles of the Rabbit, J. Physiol. **61**:113, 1926.

sodium phosphate, potassium chloride, sodium bromide, strontium chloride and magnesium chloride.

In summary, it may be stated that all easily soluble substances so far experimented with, which are not toxic enough to red blood cells to produce hemolysis or severe injury, have an inhibiting influence on the staining of reticulocytes by brilliant cresyl blue.

It may be argued that such strong concentrations of substances produce permanent changes of the membranes of the red blood cells or even "fixing" of the red blood cells, thus preventing the entrance of brilliant cresyl blue into the cells. That this is not likely is shown by the following experiment: red blood cells were left in contact with a solution of liver extract or glycine; then the cells were washed several times by centrifugation and the addition of physiologic solution of sodium chloride or buffer solution; if the cells were then stained supravitally the reticulocytes appeared as originally, and diffuse basophilia was absent. Therefore, the liver extract or glycine inhibited the staining of the reticular material only as long as the test substance remained in contact with the red blood cells.

Reticulated cells stained as usual if they were crenated by exposure to a small amount of alcohol, showing that crenation is not a factor in the inhibitive influence of these substances.

Preparations were examined in the moist state after supravital staining with brilliant cresyl blue, but without fixing and counterstaining with Wright's stain. The influence of solutions of substances was seen to be the same here as in the fixed preparations; no or few reticulocytes were observed. Under these circumstances, on gross as well as microscopic inspection of the smears, there seemed to be an inability of the dye to "take," and the red blood cells tended to remain unstained.

No definite difference between the effects on rabbit and human reticulocytes was found in these experiments. The human blood was more satisfactory because the cells withstood injury better and more perfect preparations could be made.

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF SUBSTANCES

Table 3 shows the results when different concentrations of liver extract, calcium chloride and sodium chloride were used. The inhibiting influence of these substances on the staining of reticulocytes by brilliant cresyl blue varies with the strength of the solution of the substance. This was less true of sodium chloride in concentrations higher than 2.5 per cent when the cells tended to hemolyze in the process of smearing, which rendered counting difficult. That very dilute solutions can have an influence is seen from the data in table 3 in the cases in which 0.5 per cent calcium chloride and 0.5 per cent sodium chloride were

used. It was noticed that physiologic solution of sodium chloride, or phosphate buffers, particularly if potassium were present, had a slight inhibiting influence (table 2). This was more apparent in the actual microscopic study of the preparations, in which poorly defined reticular substance or cells with diffuse basophilia and a few fine threads of reticular substance were present. These cells were counted as reticulocytes in the quantitative experiments. Preparations in which only diffusely basophilic cells and no reticulocytes were present were usually those in which very strong concentrations of substances were used.

TABLE 3.—*The Influence of Different Concentrations of Substances on the Staining of Reticulocytes (0.4 cc. of Solution Added to 0.2 cc. of Defibrinated Blood)*

Substance	Concentration of Solution, per Cent	Period of Exposure, Minutes	Reticulocytes	
			Control, per Cent	After Exposure to Solution, per Cent
Liver extract no. 343 (N.N.R.)	20.0	20	13.0	1.5
	10.0	20	13.0	4.6
	5.0	20	13.0	11.9
	1.0	20	13.0	12.8
Calcium chloride	10.0	15	21.2	0.4
	5.0	15	21.2	1.8
	2.5	15	21.2	11.8
	0.5	15	21.2	17.8
Sodium chloride	10.0	15	21.2	0.8
	5.0	15	21.2	9.8
	2.5	15	21.2	3.4
	0.5	15	21.2	20.2
Sodium chloride*	6.0	1	16.6	3.3
	4.0	1	16.6	4.9
	3.5	1	16.6	3.3
	3.0	1	16.6	6.8
	2.5	1	16.6	8.8
	2.0	1	16.6	10.8

* In this experiment 0.1 cc. of 0.4 per cent brilliant cresyl blue was added to the mixture and smears were taken one minute later, plain cover slips being used.

INFLUENCE OF SOLUTIONS OF SUBSTANCES IN CONTACT WITH RETICULOCYTES FOR VARYING PERIODS OF TIME

When an effective solution was left in contact with the cells for an hour or more, the percentage of reticulocytes tended to return to the original value; that is, the substance tended to lose its inhibiting influence on the staining of the reticular substance by the dye (table 4). This has not always been true, as in the case of dextrose. Certain preparations and certain strengths of solutions have failed to show this effect. However, the effect has repeatedly been demonstrated for liver extracts. The effect was more pronounced when the experiments were carried out at 37.5 C. than at room temperature. That this phenomenon, namely, the weakening of the inhibiting influence of substances when left in contact with cells for an hour or more, could not

be due to the gradual breaking down of amino-acids or polypeptids [16] is shown by the fact that sodium chloride acts in a manner similar to liver extract and amino-acids.

If the mixtures are left in the incubator for longer than several hours, the percentage of reticulocytes begins to diminish, a process which has been described elsewhere [2] and which is believed to be one of maturation of the reticulocytes.

COMMENT

It is assumed from these studies that the presence of diffuse basophilia in a cell indicates that the brilliant cresyl blue has not acted on the basophilic substance to change it to reticular substance. This may be due either to the inability of the dye to enter the cell or to the inability of the dye already in the cell to produce its characteristic precipitation-like action on the basophilic substance. That it is related to the permeability of the red blood cell to brilliant cresyl blue seems more probable. Both conditions are representative of extremely complicated mechanisms, and it is impossible at the present time to do more than conjecture as to the exact nature of the phenomenon. Gawrilow [8] discussed thoroughly problems concerning the "vitally" staining substance in young red blood cells and stated that a more thorough physical and colloidal chemical knowledge of this substance is needed for its better understanding.

It is unlikely that the dye is altered chemically or that it is oxidized to a colorless compound, because the inhibiting effect on the staining of reticulocytes is produced by so many different kinds of substances. Alteration in p_H has little to do with the phenomenon as a whole, although it may well modify the results to some extent. Variations in staining cells under extracellular conditions of different p_H were noted by Irwin,[17] Brooks,[18] Ochs,[19] Tolstoouhov [20] and Gawrilow.[8]

16. Hiruma, K.: On the Fate of Amino-Acids Permeated into the Red Corpuscles, Japan M. World **2**:65, 1922.

17. Irwin, M.: The Penetration of Basic Dye into Nitella and Valonia in the Presence of Certain Acids, Buffer Mixtures, and Salts, J. Gen. Physiol. **10**:271, 1926; Certain Effects of Salts on Penetration of Brilliant Cresyl Blue into Nitella, ibid. **10**:425, 1927; The Effect of Acetate Buffer Mixtures, Acetic Acid, and Sodium Acetate on the Protoplasm, as Influencing the Rate of the Penetration of Cresyl Blue into the Vacuole of Nitella, ibid. **11**:111, 1927; Counteraction of the Inhibiting Effects of Various Substances on Nitella, ibid. **11**:123, 1927.

18. Brooks, M. M.: Studies on Permeability of Living Cells: IX. Does Methylene Blue Itself Penetrate? Univ. California Publ., Zool. **31**:79, 1927.

19. Ochs, G. W.: Ueber den Einfluss der Temperatur auf die Färbung von Blutausstrich-Präparaten, Folia haemat. **37**:241, 1928.

20. Tolstoouhov, A. V.: Effect of Preliminary Treatment on Staining Properties of the Tissues, Stain Technology **3**:49, 1928.

Gawrilow considered of importance the hydrogen ion concentration of the aqueous medium in which the supravital staining of reticulocytes takes place. He placed red blood cells of guinea-pigs in Sorenson's phosphate buffer mixtures and stained them supravitally with polychrome methylene blue (methylthionine chloride, U. S. P.), counterstaining them with giemsa. In alkaline mediums an outstanding network was seen; in acid mediums very little network was observed, but much basophilia; in intermediate solutions all degrees of basophilia and network appeared. This influence of the p_H undoubtedly varies for the dye used and for the experimental conditions under which the staining takes place, for we have found that brilliant cresyl blue with a similar range of p_H stains the reticulocytes uniformly by our experimental methods. Also, phosphate buffer itself may have a certain inhibiting effect on the staining of reticulocytes.

Instances of the inhibiting influence of substances on the permeability of cell membranes are numerous in the literature. Höber and Memmesheimer [21] found that cane sugar and glycine had a prohibitive influence on the entrance of rhodamine B, methylene blue or methyl violet B into ox blood cells. In 1894, Bremer [22] and in 1897, Le Goff [23] reported poor staining of red blood cells by methylene blue in cases of diabetes, and they suggested this as a test for diabetes. The phenomenon apparently depends on the presence of increased amounts of dextrose in the blood. Loeb [24] described what he named the "antagonistic salt action." Solutions of electrolytes, such as sodium chloride and calcium chloride in certain concentration, inhibited the entrance of potassium chloride or acids into the embryo of eggs of *Fundulus*.

Brooks [25] studied the inhibiting effects of water and salts on the entrance of the dye, dahlia, into the sap of the cells of *Nitella*. She employed the following substances in the order of their power of inhibiting the entrance of the dye: distilled water, tap water, sodium chloride, potassium chloride, calcium chloride and magnesium chloride.

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21. Höber, R., and Memmesheimer, A.: Einige Beobachtungen über Permeabilitätsänderungen bei roten Blutkörperchen in Lösungen von Nichtleitern, Arch. f. d. ges. Physiol. **198**:564, 1923.

22. Bremer, L.: Ueber eine Färbemethode, mit der man Diabetes und Glycosurie aus dem Blute diagnostisieren kann, Centralbl. f. d. med. Wissensch. **32**:850, 1894.

23. Le Goff, Jean-Marie: Sur certaines reactions chromatiques du sang dans le diabète sucré, Thèse, Paris, 1897.

24. Loeb, J.: The Mechanism of the Diffusion of Electrolytes through the Membranes of Living Cells, J. Biol. Chem. **27**:339, 353 and 363, 1916; **28**:175, 1916.

25. Brooks, M. M.: Studies on the Permeability of Living Cells: VIII. The Effect of Chlorides upon the Penetration of Dahlia into Nitella, Protoplasma **2**:420, 1927.

Irwin [17] found that sodium and potassium ions, phosphoric acid, hydrochloric acid, phosphate and acetate buffers and acetic acid inhibited the rate of penetration of brilliant cresyl blue into *Nitella*. A direct analogy between Brooks' and Irwin's experiments and those regarding the permeability of the membranes of the red blood cells should not be drawn, however, because in the latter case, at least, there is doubt whether we are dealing with vital phenomena (Nittis [26]). Pearse [27] found that the reticulum of immature red blood corpuscles in solutions of the same p_H but of different composition reacted to neutral red in the same way as the stained structures of dead leukocytes, and the opposite to the structures within living white corpuscles. He gave this as evidence in favor of the view set forth by Key that the reticulum is an inactive substance, but he stated that since erythrocytes are not considered cytologically as true cells the semipermeable properties of their membrane may not be comparable to those of other living cells.

Kerr [28] noted that red blood cells become progressively more permeable to cations as the proportion of serum present decreases. He remarked that 0.85 per cent sodium chloride was not "physiologic" since it permits the transfer of ions. This opens an important lead on the problems presented here. Physiologic solution of sodium chloride inhibits the staining of reticulocytes by brilliant cresyl blue. Other substances that were employed in strong concentrations were certainly not "physiologic." That changes take place under these conditions, undoubtedly in the transfer of ions, is probable, as is shown in the experiments given in table 4, in which the inhibiting influence of the solutions is shown to diminish with time. Neuhausen and Breslin [29] and Mond and Gertz [30] studied problems related to the permeability of the membranes of red blood cells which bear especial relation to the phenomena presented in this paper.

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26. Nittis, S.: "Supra-Vital" Staining Phenomena of Erythrocytes, Folia haemat. **41**:385, 1930.

27. Pearse, H. E.: The Permeability of Human Blood Cells to Carbon Dioxid and Ammonium Hydroxid in Solutions of the Same p_H, Arch. Int. Med. **35**:347 (March) 1925.

28. Kerr, S. E.: Studies on the Inorganic Composition of Blood: III. The Influence of Serum on the Permeability of Erythrocytes to Potassium and Sodium, J. Biol. Chem. **85**:47, 1929.

29. Neuhausen, B. S., and Breslin, J. E.: Study of the Influence of Chemicals on Erythrocyte Membranes by Changes in Corpuscular Volume, Bull. Johns Hopkins Hosp. **34**:199, 1923.

30. Mond, R., and Gertz, H.: Vergleichende Untersuchungen über Membranstruktur und Permeabilität der roten Blutkörperchen verschiedener Säugetiere, Arch. f. d. ges. Physiol. **221**:623, 1929.

It seems unlikely that red blood cells are the only cells that contain a substance which is closely connected in some way with the maturation of cells, and which is demonstrable by certain staining methods. Unpublished data at hand indicate that a substance similarly demonstrable is present also in the white blood cells and bears a relation to the maturation changes of these cells. Therefore, it seems reasonable to conclude that studies of the sort presented in this paper tend to emphasize general, rather than special, phenomena of cell life. As knowledge

TABLE 4.—*The Influence of Solutions of Substances in Contact with Blood for Some Hours on the Staining of Reticulocytes (0.4 cc of Solution Added to 0.2 cc. of Defibrinated Blood)*

Substance	Period of Exposure	Percentage of Reticulocytes	
		Control Blood	After Exposure to Solution
10 per cent liver extract no. 343 (N.N.R.)	15 minutes	34.2	10.5
	50 minutes	....	25.7
	3 hours, 15 minutes	....	31.0
10 per cent liver extract no. 343 (N.N.R.)	15 minutes	34.2	23.7
	3 hours, 15 minutes	....	25.0
10 per cent liver extract no. 343 (N.N.R.)	15 minutes	34.2	19.1
	3 hours, 15 minutes	....	23.4
2.5 per cent sodium chloride	5 minutes	29.4	0.4
	2 hours	....	25.8
8 per cent dextrose	5 minutes	34.3	2.2
	1 hour	....	1.6
	2 hours	....	1.0
	3 hours	....	0.9
8 per cent dextrose*	5 minutes	29.8	0.8
	1 hour	....	2.1
	2 hours	....	1.5
2.5 per cent sodium chloride*	5 minutes	33.8	21.0
	15 minutes	....	17.1
	1 hour	....	21.4
	3 hours	....	18.9
2.5 per cent sodium chloride†	15 minutes	24.2	11.0
	1 hour	....	12.6
	3 hours	....	15.7
	6 hours	22.9	12.6

* These experiments were carried out at room temperature; all of the other experiments were carried out at 37.5 C.

† In this experiment 0.1 cc. of 0.4 per cent brilliant cresyl blue was added to the mixture of cells and solution, and smears on glass cover slips were taken after one minute.

concerning cellular biology accumulates it will unfold the secrets of vital processes and lead eventually to the defeat of disorders associated with the abnormal growth of cells.

CONCLUSIONS

1. A variety of substances in solution, including liver extracts, amino-acids and certain salts, have an inhibiting influence on the staining of reticulocytes by brilliant cresyl blue.

2. The influence of the liver extracts on the staining of reticulocytes is not related to the potency of the extracts for producing remission in pernicious anemia.

3. The inhibiting effect of a substance on the staining of reticulocytes varies with the concentration of the solution of the substance.

4. When solutions of liver extract, amino-acids or sodium chloride remain in contact with the cells for a long time, the inhibiting influence on the staining reaction tends to diminish.

5. The experiments give further evidence in support of the theory that basophilic substance and reticular substance are different manifestations of the same material.

6. The inhibiting influence on the staining of reticulocytes is assumed to be due to alteration in the permeability of the red blood cells produced by the action of various substances in solution.

PARAVERTEBRAL INJECTIONS OF ALCOHOL FOR THE RELIEF OF CARDIAC PAIN

A REVIEW OF EXPERIENCE TO DATE AND A REPORT OF NINE CASES *

ROBERT L. LEVY, M.D.
AND
RICHMOND L. MOORE, M.D.
NEW YORK

As experience accumulates following the introduction of a new therapeutic procedure, there comes a time when it is profitable to halt activity and critically appraise the value of what has been accomplished. Following such an analysis, ineffective measures are discarded, modifications often suggest themselves, and plans for further development of the method may be formulated. Cervical sympathectomy as a means of alleviating pain of cardiac origin has not stood the test of trial, and in America it has fallen into disuse. The many types of operation employed and the variability of results afford abundant evidence that ablation of any portions of the sympathetic chain in the neck cannot interrupt all of the nervous pathways concerned. Furthermore, the most intense suffering is encountered in patients with coronary disease, and as a rule it is inadvisable to submit such persons to an extensive surgical operation. When discomfort is so great that life becomes burdensome, any measure offering a reasonable hope of relief deserves serious consideration and trial under suitably controlled conditions. It has seemed to us timely to survey the results to date of paravertebral injections of alcohol and to record personal observations in nine cases.

REVIEW OF THE LITERATURE

The idea of paravertebral alcohol block for the relief of cardiac pain was the outcome of a logical sequence of events. Paravertebral injections of procaine hydrochloride have been used for a number of years in order to induce local anesthesia for the performance of surgical operations. In Germany, the method has been employed more recently for the differential diagnosis of various abdominal conditions by abolishing pain in segmental areas and thereby deducing its source of origin. At

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* From the Departments of Medicine and Surgery, College of Physicians and Surgeons of Columbia University and the Presbyterian Hospital.

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the suggestion of von Bergmann, Laewen [1] first practiced the injection of procaine hydrochloride into the dorsal rami in a case of angina pectoris, but gave no detailed account of the patient or of the effects. Brunn and Mandl,[2] stimulated by Pal, then undertook to study the method systematically from a therapeutic point of view. Luger,[3] Pal,[4] Mandl,[5] Brunn [6] and others followed with reports of series of cases. After tabulating and analyzing this literature, it became apparent that the injections were made into various nerves at different cervical and thoracic levels; the clinical histories were recorded only briefly, and the relief afforded, though definite as a rule, was of relatively short duration. However, Mandl stated that in three instances the attacks disappeared for from six months to a year.

Swetlow [7] suggested the injection of alcohol following that of procaine hydrochloride, with a view of inducing Wallerian degeneration in the fibers of the rami communicantes and thereby insuring greater permanence of relief. He undertook to map out areas of disturbed cutaneous sensibility in his patients, making injections into the roots corresponding to the segmental distribution of sensory changes. In a series of papers, Swetlow reported twenty-two cases.[7] Nineteen cases from the Massachusetts General Hospital have been recorded at various times by White and White,[8] Sprague and Mixter,[9] Richardson and White,[10]

1. Laewen, A.: Paravertebrale Novokaininjektionen zur differential Diagnose intraabdomineller Erkrankungen, Zentralbl. f. Chir. **49**:1510, 1922.

2. Brunn, F., and Mandl, F.: Die paravertebrale Injektion zur Bekämpfung visceraler Schmerzen, Wien. klin. Wchnschr. **37**:511 (May 22) 1924.

3. Luger, A.: Therapeutischer Versuch bei Aortalgie, Wien. med. Wchnschr. **74**:2543 (Nov. 22) 1924.

4. Pal, J.: Zur Aussprache über Klinik der Angina pectoris, Wien. klin. Wchnschr. **37**:351 (April 3) 1924.

5. Mandl, F.: Weitere Erfahrungen mit der paravertebralen Injektion bei der Angina pectoris, Wien. klin. Wchnschr. **38**:759 (July 2) 1925.

6. Brunn, F.: Zur Behandlung der Angina pectoris mit paravertebralen Injektion, Wien. klin. Wchnschr. **39**:110 (Sept. 23) 1926.

7. Swetlow, G. I.: Paravertebral Alcohol Block in Cardiac Pain, Am. Heart J. **1**:393 (April) 1926. Swetlow, G. I., and Schwartz, S. P.: The Treatment of Cardiac Pain by Paravertebral Alcohol Block, J. A. M. A. **86**:1679 (May 29) 1926. Swetlow, G. I.: A Clinicophysiologic Study of the Pathway of Pain Impulses in Cardiac Disease, Am. J. M. Sc. **178**:345 (Sept.) 1929; Angina Pectoris: Paravertebral Alcohol Block for the Relief of Pain, Am. J. Surg. **9**:88 (July) 1930.

8. White, J. C., and White, P. D.: Angina Pectoris: Treatment with Paravertebral Alcohol Injections, J. A. M. A. **90**:1099 (April 7) 1928.

9. Sprague, H. B., and Mixter, W. J.: A New Method for the Treatment of Severe Angina Pectoris, New England J. Med. **200**:199 (Jan. 24) 1929.

10. Richardson, E. P., and White, P. D.: Sympathectomy in the Treatment of Angina Pectoris: Comparison of Results with Those from Paravertebral Alcohol Injection, Am. J. M. Sc. **177**:161 (Feb.) 1929.

Mixter and White [11] and White.[12] Pletnew and Hesine [13] reported the administration of seventy-three injections in eighteen patients, and Cattell and Hurxthal [14] published the account of another case from the Lahey Clinic in Boston. Including the nine cases in our own series, there are available records of sixty-eight patients who received injections of alcohol.

ANATOMY AND PHYSIOLOGY OF THE NERVES CONCERNED IN THE MECHANISM OF CARDIAC PAIN

Knowledge concerning the nervous pathways by which painful impulses are transmitted from the heart and aorta to the central nervous system is still incomplete. Certain facts have been established with reasonable certainty, and in this brief summary the attempt will be made to remain within the domain of probability rather than to wander in the realm of the possible. The following statements, as well as the accompanying diagram (fig. 1), are based largely on the work of Langley,[15] Edgeworth [16] and Ranson.[17]

In the words of Ranson:

> Whether or not the intense pain which characterizes the anginal attacks is due to stretching of a diseased aorta, to spasm of the coronary arteries, or to anemia of the myocardium, the pain in itself clearly indicates that there is an irritation of sensory fibers in the heart wall or in the immediately associated arteries.

These sensory fibers form a plexus surrounding the coronary vessels and aorta, and in this plexus minute sympathetic ganglions are found. The course of the sensory fibers for the heart and its associated arteries is fairly well known (fig. 1). Afferent impulses are carried to the

11. Mixter, W. J., and White, J. C.: Alcohol Injection in Angina Pectoris, Ann. Surg. **89**:199 (Feb.) 1929.

12. White, J. C.: Angina Pectoris: Relief of Pain by Paravertebral Alcohol Block of the Upper Dorsal Sympathetic Rami, Arch. Neurol. & Psychiat. **22**:304 (Aug.) 1929; Angina Pectoris: Treatment by Paravertebral Alcohol Injection or Operation Based on the Newer Concepts of Cardiac Innervation, Am. J. Surg. **9**:98 (July) 1930.

13. Pletnew, D. D., and Hesine, V. R.: Paravertebral Injections for Angina Pectoris, Klin. med. **6**:797, 1928. (The original of this paper was not available. A translated abstract was obtained from the Surgeon-General's Library, Washington, D. C.). Pletnew, D. D.: Die Anwendung systematischer paravertebraler Injektionen bei der Behandlung der Angina Pectoris, Med. Welt **4**:848 (June 14) and 884 (June 21) 1930.

14. Cattell, R. B., and Hurxthal, L. M.: Paravertebral Alcohol Injection for Angina Pectoris, J. A. M. A. **92**:1519 (May 4) 1929.

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16. Edgeworth, F. H.: On a Large-Fibred Sensory Supply of the Thoracic and Abdominal Viscera, J. Physiol. **13**:260, 1892.

17. Ranson, S. W.: The Cardiac Nerves in Angina Pectoris, Am. Heart J. **1**:508 (April) 1926.

spinal cord along the cardiac branches of the middle and inferior cervical sympathetic ganglions (middle and inferior cardiac nerves) and pass through the sympathetic trunk and into the upper five (possibly six) thoracic nerves through the corresponding white rami communicantes. Thus, in accordance with Head's [18] demonstration that the pain of visceral disease is referred to the cutaneous segmental areas in the distribution of the nerves supplying a viscus, pain of cardiac origin is referred chiefly to the wall of the chest and the inner aspect of the left arm,

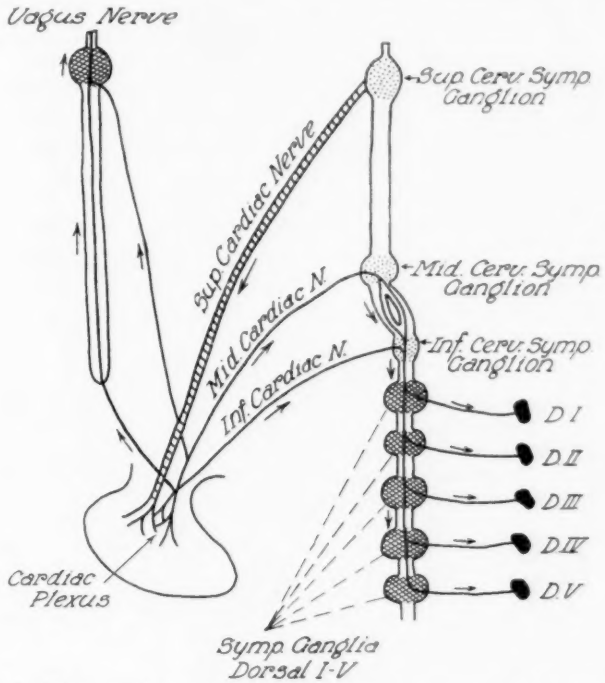

Fig. 1.—Diagram showing the nervous pathways concerned in the origin and transmission of cardiac pain. Note that the superior cardiac nerve conducts only motor impulses.

corresponding to the distribution of the first five dorsal nerves. Afferent impulses from the heart are also carried to the brain along the vagus and may cause pain referred to the head through communicating branches to the third, fourth and fifth cranial nerves.

No afferent fibers from the heart to the superior cervical sympathetic ganglion have been demonstrated, and for the present the superior

18. Head, H.: On Disturbances of Sensation, with Especial Reference to the Pain of Visceral Disease, Brain **16**:99, 1893; **19**:153, 1896.

cardiac nerve must be regarded as entirely motor. In certain cases, particularly those of Coffey and Brown,[19] relief from painful paroxysms has been obtained following the removal of only the superior cervical sympathetic ganglion. To explain this observation, Holmes and Ranson [20] have advanced the hypothesis that vasoconstrictor fibers reach the cardiac plexus from this ganglion by way of the superior cardiac nerve. Under these conditions, the painful seizures are due to coronary spasm. This mechanism might well explain the paroxysms in patients showing at necropsy no morbid changes in the heart or in its vascular bed, as exemplified by the case reported by one of us (Levy [21]).

In view of the number of afferent pathways from the heart to the cord and the brain and with the possibility of individual variation from a standard pattern being taken into account, the difficulty of interrupting all of these paths by cervical sympathectomy becomes apparent. On theoretical grounds, paravertebral injections of alcohol should afford a better chance for success with less trauma, as they interrupt the passage of painful impulses at their point of entrance to the spinal cord. However, impulses can still be transmitted to the cranial nerves through the vagus, and vasoconstriction of the coronaries may still be possible via the superior cardiac nerve. Probably other uncharted pathways are concerned.

TECHNIC OF PARAVERTEBRAL INJECTIONS

The technic employed by previous workers as well as by us is the one described by Labat.[22] It has been given in detail by Swetlow,[23] and certain slight modifications have been suggested by White and White.[8] No attempt was made in our patients to determine into which roots injections should be made by mapping out areas of disturbed sensibility of the skin. It was frequently impossible to delimit definite levels, and it was believed that by making injections into the first five dorsal rami, the main known sensory pathways to the cord were interrupted.

In all of our cases injections were made in the same way. The patient was placed on the right side, with knees bent and drawn toward the chin. The skin was prepared with iodine, and the spines of the first five dorsal vertebrae were marked with acriflavine. Dots were made 4 cm. to the left of each dorsal spine, and the skin was infiltrated with a 1 per cent solution of procaine hydrochloride at each of these points. A needle 8 or 10 cm. in length was inserted through each wheal, perpendicularly to the skin, until it touched the border of the underlying rib. The direction of the needle was then shifted so that it pointed mesially

19. Coffey, W. B., and Brown, P. K.: The Surgical Treatment of Angina Pectoris, Arch. Int. Med. **31**:200 (Feb.) 1923.

20. Holmes, W., and Ranson, S. W.: Cervical Sympathectomy in Angina Pectoris, J. Lab. & Clin. Med. **10**:183 (Dec.) 1924.

21. Levy, R. L.: Cardiac Pain: A Consideration of Its Nosology and Clinical Associations, Am. Heart J. **4**:377 (April) 1929.

22. Labat, G.: Regional Anesthesia: Its Technique and Clinical Application, Philadelphia, W. B. Saunders Company, 1922.

23. Swetlow (footnote 7, first reference).

at an angle of 45 degrees; it was then pushed in 2 cm. farther. In the early cases, a water manometer was connected with the needle, to ascertain whether the tip was within the pleural cavity. If oscillations corresponding to respiration were observed, the needle was withdrawn and reinserted. Next 5 cc. of a 1 per cent solution of procaine hydrochloride was injected through each needle. After an interval of ten minutes, 5 cc. of an 80 per cent solution of alcohol was injected through each needle, and as soon as injection was completed, the needle was withdrawn.

According to White,[12] the injection of 5 cc. of alcohol causes the development of an area of necrosis, followed by fibrosis, that is only about 1 cm. in diameter, whereas 5 cc. of procaine hydrochloride diffuses over a somewhat wider area. In order to infiltrate with alcohol the regions through which the rami communicantes pass, it is clearly imperative that the injections be made accurately. The procedure is relatively simple, but it requires practice on the cadaver.

REPORTS OF PERSONAL CASES

CASE 1.—*History.*—Nathan Z., aged 36, a baker, was first seen in the Cardiac Clinic on April 16, 1928. He had been remarkably well up to the onset of the present illness. In August, 1928 (eight months previously), while walking home from work, he had a sudden attack of pain in the left side of the chest which radiated to the left arm and was accompanied by a sense of choking and oppression. He fell on the street and had to be taken home. The pain persisted throughout the night, and it was relieved the following morning by hypodermic medication. The patient remained in bed for three weeks. Another attack occurred in November, 1928, which again confined him to bed for two weeks. Since then, walking for even a short distance or any excitement induced an attack. He described the pain as beginning on the inner aspect of the left elbow and traveling upward along the arm to the precordium. The patient also had attacks of pain at night. Inhalation of amyl nitrite afforded relief. He had been unable to work for eight months.

Examination.—On examination, the heart was found to be slightly enlarged to the left. The rhythm was regular. The sounds were rather weak and had a tic-tac quality, but a gallop rhythm was not heard. The blood pressure was 134 systolic and 86 diastolic. An electrocardiogram showed inversion of T_1 and notching of R_3. A teleoroentgenogram of the heart showed moderate enlargement with tortuosity of the aorta and prominence of the knob. The diagnosis was arteriosclerosis, coronary sclerosis, dilatation of the aorta and old coronary thrombosis, with a healed myocardial infarct.

Operation.—The patient was admitted to the hospital on May 6, 1929. He was kept in bed for two weeks, during which he had several mild attacks of pain. He was then allowed to be up and about the ward for a week, and his symptoms increased. A paravertebral injection of alcohol was made on May 31, 1929. There was slight discomfort during the operation, and at its conclusion he was in good condition. Horner's syndrome (miosis, ptosis and enophthalmos) was not noted.

Postoperative Course.—On the evening after the operation was performed, the temperature rose to 101.2 F. and the heart rate to 110. On the following day the temperature was 101.8 F., and a slight elevation ranging from 100 to 101.2 F. persisted for ten days. Tachycardia was present for only three days. On the

day of injection the blood pressure was 145 systolic and 95 diastolic. On the next day it was 105 systolic and 75 diastolic, and then it gradually resumed its former level. There were slight variations in the degree of inversion of T_1 and in the location of the notch in R_3, but these were not striking. The sensory disturbances are shown in the accompanying chart (fig. 2). The patient vomited once and complained of severe headache. He complained of pain in the left side of the chest, which was aggravated by respiration. In the course of a week almost complete anesthesia of the skin corresponding to the first five thoracic segments, in front and in back, was observed. He continued to complain of pain in the upper part of the left side of the chest, but there was no recurrence of the original

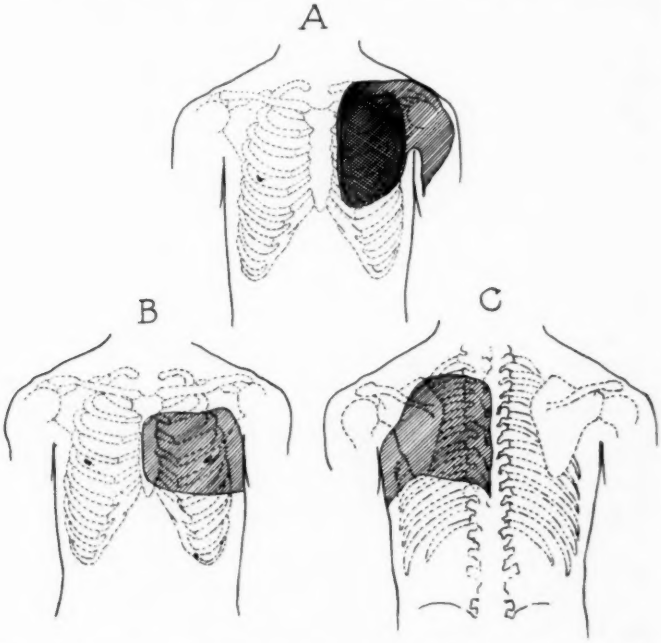

Fig. 2.—Sensory disturbances in case 1. *A*, before injection. The single cross hatching denotes hyperesthesia to touch and pain; the double hatching, hyperesthesia to heat and to cold. *B* and *C*, one day after injection of the first five dorsal rami. The hatched areas denote hypesthesia to touch, pain, heat and cold.

attacks. On June 19, he walked briskly four times the length of the hospital corridor without discomfort.

The patient was seen at frequent intervals. For six weeks, he continued to complain of pain in the region of the injections, which radiated around the chest to the front, but the precordial discomfort never recurred. There was a slight "substernal heaviness" on walking rapidly. On November 18 (six months after injection), he returned to his work as a baker. His employer excused him from lifting heavy sacks of flour. At this time the heart rate was 90, and the

rhythm was regular. The blood pressure was 142 systolic and 96 diastolic. In March, 1930 (ten months after injection), he complained of dyspnea on walking in the street. He was given a course of digitalis, with much benefit. The last follow-up was on October 2, sixteen months after the injection. He had experienced no pain, but he still became slightly short of breath on walking rapidly. He was working regularly as a baker.

CASE 2.—*History.*—Benjamin B., aged 54, a hatmaker and porter, was known to have had diabetes for at least three years. He first noticed cardiac pain in 1925, four years before examination. The attacks were precordial and radiated to the left shoulder and down the left arm; they were definitely related to exertion. During the attacks he became dyspneic and sweated profusely. The diabetes was satisfactorily controlled. Over a period of three years, various therapeutic measures were tried for relief from pain, including deep roentgen therapy administered to the chest, but there was no improvement and he was unable to work.

Examination.—The patient was admitted to the hospital on Oct. 23, 1929, for the paravertebral injection of alcohol. The diagnosis was diabetes mellitus, arteriosclerosis with coronary involvement and cardiac hypertrophy. The heart was moderately enlarged. The cardiac rhythm was regular, and the sounds were of moderate intensity. The blood pressure was 135 systolic and 74 diastolic. The Wassermann reaction of the blood was negative. The electrocardiogram showed no significant changes. Roentgenograms of the legs showed calcification of the blood vessels.

Operation and Course.—A paravertebral injection of alcohol was given on October 29. The patient complained of mild local discomfort at the time of injection. Horner's syndrome did not develop. On the day following injection, the temperature rose to 102.2 F. and the heart rate to 108. There was a slight elevation in temperature for five days; afterward both fever and tachycardia subsided. The patient complained of great pain in the left side of the back at the site of injection, particularly on moving the left arm. There was a small area of hyperesthesia and sensitiveness to cold on the left side of the neck, which extended from the chin to the angle of the jaw. There was no recurrence of the attacks. Ten days after injection, he again complained of substernal and precordial pain, chiefly at night; it was sometimes associated with nausea and vomiting. It was not like the pain from which he formerly suffered. Three weeks after injection he experienced his first attack of precordial pain, which shot down the left arm and caused numbness of the index and middle fingers of the left hand. He was still in bed at this time. He was discharged from the hospital on November 22, three and one-half weeks after the injection. At this time he was free from cardiac discomfort. There were no changes in the form of the electrocardiogram.

On December 18, he was still experiencing cardiac pain, but his condition was better than before injection. On Jan. 13, 1930 (two and one-half months after injection), he stated that the attacks were recurring and the dyspnea was increasing. At this time, it seemed probable that gradual arteriosclerotic closure of the left coronary was taking place.

Readmission to Hospital.—The patient was readmitted to the hospital on January 13, because of a recurrence of the pain in the left side of the chest. He looked poorly. The blood pressure was 145 systolic and 70 diastolic. There was still hyperesthesia and pain in the area supplied by the injected rami communicantes. It appeared likely that complete degeneration of the nerve roots had not occurred and that the painful phenomena were due to persistent irritation. Reinjection was advised but refused. The attacks now were not relieved by glyceryl

trinitrate. The patient became dizzy and somewhat confused mentally, so that it was difficult to estimate the degree of suffering. The duration of follow-up was only four months. On February 11, it became necessary to transfer him to another hospital for permanent care. It was thought that the cerebral symptoms were due to progressive arterial changes. The patient was afforded complete relief for about three weeks and partial relief for three months.

CASE 3.—*History.*—Sarah Z., aged 68, a Polish housewife, was admitted to the hospital on April 19, 1929. She had had pneumonia six years before, which was followed by an abscess in the left hip that had resulted in shortening of the leg. Cholecystectomy had been done twenty-five years previously. Diabetes was discovered during the course of pneumonia. For twenty-five years, she had noted dyspnea on exertion, together with cramplike pain in the precordia. Lately the pain had radiated to the left arm. Glyceryl trinitrate afforded relief. An attack lasted for as long as an hour. Recently she had noted swelling of the ankles, and she was obliged to sleep on several pillows.

Examination.—The patient was obese. The lungs were emphysematous. The heart was slightly enlarged to the left, and roentgen examination showed that the aortic knob was prominent. The heart sounds were rather weak, and there was a systolic blow at the apex. The rhythm was regular. The blood pressure was 140 systolic and 75 diastolic. The electrocardiogram showed only slight left ventricular preponderance. The diagnosis was diabetes mellitus and coronary sclerosis.

Course.—The diabetes was readily controlled. While in bed in the ward, the patient had several severe attacks of pain, which radiated to the neck and down the inner side of the left arm and lasted from ten minutes to one-half hour. She cried, perspired and tossed about. During the attack the blood pressure rose to 230 systolic and 130 diastolic. Amyl nitrite and glyceryl trinitrate afforded only slight relief, and morphine was necessary to make her comfortable. There was an area of marked hyperesthesia to pain, touch, heat and cold, covering the left side of the head, the neck, the left side of the chest and the abdomen, in front and in back. The area of greatest intensity was in the lower part of the precordia and the left axilla and to a lesser degree over the left temple and in the region of the left mandible.

Operation.—On June 15, a paravertebral injection of alcohol was given. The patient stood this procedure very well. She experienced severe pain in the precordial region when alcohol was injected into the second dorsal root. On the day following operation, the temperature rose to 100.4 F. and the heart rate to 112, but next morning both the temperature and the pulse rate were normal. For two days there was moderate pain at the site of the injections, radiating around the left side of the chest. Five days after the injection, she awoke suddenly with a choking sensation and pain in the precordia, which radiated to the shoulder, the left side of the jaw and into the arm. During the attack, the blood pressure was 164 systolic and 104 diastolic. Inhalation of amyl nitrite gave no relief, but morphine made her comfortable. She had another attack on the tenth day after operation, and again on the twelfth, each time with elevation of blood pressure. During the height of the last attack the blood pressure was 210 systolic and 150 diastolic. She stated that it felt as though the heart were being "squeezed like a ball." She was discharged from the hospital on June 28.

Postoperative Course.—The patient was seen on September 30 (three months after injection). Her daughter stated that she had not had an attack for seven weeks, and that "she was a different woman since the operation." The patient

however, maintained that she was still having pain, which began in the ring finger and the little finger of the left hand, traveled up the arm to the neck and terminated in a sense of substernal oppression with choking. The pain was unrelated to effort. Walking rapidly or going up stairs caused dyspnea, and she felt that she was not improved. The blood pressure was 158 systolic and 94 diastolic. The heart rate was 92. The heart sounds had a tic-tac, valvular quality. There was slight edema of the ankles and legs.

Readmission.—The patient was readmitted to the hospital on November 12, because of a recurrence of the cardiac attacks, symptoms of cardiac insufficiency and asthma. The paroxysms recurred while the patient was in the hospital, and she became irrational and violent. It was thought that the mental disturbance was due to progressive cerebral arteriosclerosis. On December 11 (seven months after injection), she was transferred to a psychopathic hospital. In this case there was probable partial relief for seven weeks, followed by recurrence of the attacks.

CASE 4.—*History.*—Samuel L., aged 35, a cutter of boxes, was first seen in the Cardiac Clinic on Feb. 19, 1930. He had had rheumatic pains and swelling of the joints at intervals for four years, which on one occasion had confined him to bed for a week. The present illness had begun eight weeks previously with substernal pain, which radiated upward symmetrically to both sides of the chest and the neck and to the jaws. On one occasion, there was radiation to the right arm below the elbow. The attacks were induced by slight effort and lasted from a few minutes to several hours; they were only slightly relieved by glyceryl trinitrate. The patient was unable to work at his trade because of discomfort.

Examination.—The patient was rather pale, but there was no dyspnea or cyanosis. The heart was not enlarged; the rhythm was regular, and the sounds were of good quality. There was a short systolic blow at the apex and at the aortic area. The second aortic sound was slightly accentuated. The blood pressure was 142 systolic and 98 diastolic. There were numerous carious teeth, and the tonsils appeared to be badly infected. An electrocardiogram showed well marked left ventricular preponderance and diphasic T waves in leads 1 and 2. A teleoroentgenogram of the heart showed slight cardiac enlargement due to prominence of the left ventricle. The Wassermann reaction of the blood was negative. The diagnosis was coronary sclerosis, chronic tonsillitis and dental caries.

The patient was admitted to the hospital on February 26. The infected teeth were removed. Tonsillectomy was performed on March 28. In taking daily electrocardiograms, it was interesting to note slight variations in the T wave, particularly in lead 1. This wave changed from the inverted to the upright position, although no drugs were given that could have modified its form. On April 7, the patient reported that he had had no pain or discomfort since leaving the hospital, although he lived up two flights of stairs; however, on April 21 (two weeks later), he returned to the clinic, stating that he was again very uncomfortable. Walking induced severe pain and caused him to "crumple up." The discomfort was more intense than before. The pain was substernal and radiated around the axilla to the left scapular region and to the left arm. He was unable to work, and he was readmitted to the hospital on April 22. Even with rest in bed, a sense of pressure persisted in the region of the heart, although there was no acute pain. Lumbar puncture yielded normal fluid that gave a negative reaction to the Wassermann test.

On April 29, a paravertebral injection of alcohol was given. The patient complained of the usual amount of discomfort, but he did not cough or manifest any evidence of respiratory embarrassment. On May 1, two days later, he complained of sharp pain in the left side of the chest on inspiration. The temperature was 101.2 F. There were signs of fluid at the base of the left pleural cavity, which was confirmed by roentgen examination. The horizontal level of the fluid suggested to Dr. Reeves atelectasis of the lower part of the left lobe, but pneumothorax could not be seen in the upper portion of the lung. At this time the leukocytes numbered 9,400, 53 per cent of which were polymorphonuclears. The signs of fluid disappeared in the course of a week. There was a slight elevation of temperature to 100.4 F. for five days. The patient was sent home on May 10, twelve days after the injection of alcohol. He was free from pain of cardiac origin, but he complained of discomfort in the region of the injection and in the axilla.

Course.—The patient was next seen on May 26 (four weeks after injection). He still complained of intercostal pain and discomfort on breathing; the latter symptom was relieved by the placing of a tight band around the thorax. He stated that this pain was not the same kind as that which he had experienced before the injection was made. On June 9 (six weeks after injection), he still had some discomfort over the left side of the chest; the pain was constant, but it was aggravated by walking. He said that it "felt more like the pleurisy pain." He was gaining weight and feeling better. The blood pressure was 118 systolic and 82 diastolic. On June 30 (two months after injection), he said that he was feeling wonderfully well. He was entirely free from pain, and he could walk six or seven blocks slowly without discomfort and was ready to take a light job. He failed to report for further observation, and attempts to locate him were unsuccessful.

In this patient, pleural effusion was observed following the paravertebral injection of alcohol. At the same time there was fever but not leukocytosis. There were the usual radicular pain and hyperesthesia, but complete relief from the attacks of cardiac pain was obtained.

CASE 5.—*History.*—Harris C., aged 48, a restaurant keeper, was admitted to the hospital on March 22, 1930, complaining of precordial pain and dyspnea on exertion for the past nine years. He had had scarlet fever as a child, but otherwise he had been well. The present illness had begun nine years previously with pain in the left arm, which occurred in cold weather and was relieved by warmth. Within a short time, pain began to spread to his heart, and at times he noted it beneath the left eye. For the past five months he had become definitely worse. The pain was related to exertion and was relieved by nitrites. Recently, it had radiated to the shoulder, down the left arm and often to the neck, both sides of the face and the teeth. At times attacks occurred at night. During the preceding five months he had been totally incapacitated, and he had lost 35 pounds (15.9 Kg.).

Examination.—Examination showed slight cardiac enlargement to the left. The rhythm was regular. The sounds were rather weak. There was no accentuation of the aortic second. The blood pressure was 142 systolic and 70 diastolic. The electrocardiogram showed only left ventricular preponderance. The Wassermann reaction of the blood was negative. The basal metabolic rate was +12 per cent. The diagnosis was coronary sclerosis and cardiac hypertrophy.

The patient was discharged on March 28, but he was readmitted on April 14 because of the persistence of symptoms. He was taking about ten tablets of

glyceryl trinitrate during the day and using two amyl nitrite pearls at night. Walking for a distance of one-half block caused dyspnea. He was an absolute invalid, and was willing to submit to any procedure for relief.

An injection of alcohol was given on April 17. The operation went smoothly, and there was no complaint of discomfort. On the day following injection, the temperature rose to 103 F. and the heart rate to 128, and he had a sharp chill. He complained of severe pain in the left side of the chest; he coughed occasionally and said that the pain was aggravated on taking a deep breath. There were signs of fluid in the left pleural cavity. There was also definite hypesthesia of the left side of the chest, in front and in back, together with a well marked Horner's syndrome. Fluoroscopic examination of the chest showed a small amount of fluid in the left costophrenic angle. The leukocyte count was 11,600, with 86 per cent polymorphonuclears. On the second day following injection the temperature was 101.6 F.; the heart rate, 120. The blood pressure was 124 systolic and 72 diastolic. The pain on respiration was less marked.

Course.—During the following week the effusion in the left pleural cavity was absorbed. The patient complained that the left side of the chest felt dead. The temperature returned to normal on the fourth day. The patient was allowed to get up gradually. There was no return of cardiac pain, but a sense of pressure over the precordia persisted. He was seen on May 19, and again on June 2, the latter date being six weeks after injection. The pain was not as severe as before, but he still had nocturnal attacks. Any exertion caused dyspnea and pressure over the lower part of the sternum, together with a choking sensation. The arm hurt when he moved it, and there was a tender spot below the left nipple. The functional capacity was unchanged, although there was some modification of the character of the symptoms. He was unable to do any kind of work, and he felt that he had been little benefited by the injection. During the following three months, he was almost entirely free from pain. The attacks then recurred, and on October 6 (six months after injection), the condition was essentially as when the patient was first seen.

Case 6.—*History.*—Abraham G., aged 68, a retired Austrian Jew, had been observed in the outpatient department since October, 1924, and he had received treatment for diabetes mellitus and syphilis. He was admitted to the hospital on Feb. 5, 1930, because of pain in the precordia. This pain had been present for about eight years. It was almost constantly present as a dull ache and a sense of oppression, but it was aggravated by any exertion. There was no dyspnea or edema.

Examination.—The heart was slightly enlarged. The rhythm was regular; the sounds were of fairly good quality. There was no gallop rhythm and no accentuation of the second aortic sound. The peripheral vessels showed moderate thickening. The lungs were emphysematous. The blood pressure was 165 systolic and 60 diastolic. The Wassermann reaction of the blood was 4 plus. There were no signs of aortitis. The electrocardiogram showed slight left ventricular preponderance. The diagnosis was coronary sclerosis, diabetes mellitus, emphysema of the lungs and syphilis.

Operation.—An injection of alcohol was given on February 12. The patient complained of considerable discomfort during the injection, but he was in good condition at the termination of the operation. On the day following injection, the temperature rose to 101 F. and remained elevated for eight days. There was also moderate tachycardia, and the heart rate ranged from 88 to 104. Following the injection the patient complained bitterly of pain in the back, radiating around

the side of the chest. It was difficult to gage the severity of the symptoms, as he was a highly nervous person who varied his story from day to day. He left the hospital on March 2.

Course.—On March 10 (one month after injection), the patient stated that the substernal pain was somewhat less severe, although he had had an occasional twinge just to the right of the midline. The pain at the site of the injection was intense and constant, and he stated that he was unable to sleep. It appeared that the pain of cardiac origin was partially relieved, but that neuritis was still causing considerable discomfort. On October 6 (seven and one-half months after injection), he stated that he was no better than before operation. This case must be considered a therapeutic failure. Antisyphilitic treatment was resumed.

Case 7.—*History.*—Anna W., aged 50, a Polish housewife, was admitted to the hospital on March 12, 1930. She had been complaining of pain in the region of the heart for three and one-half months. Pain began suddenly while she was walking and radiated from the precordial region to the inside of both arms, to the ears and to the gums. The pain was very severe, and it was accompanied by weakness, palpitation and fear of death. The first attack lasted only a few minutes, but several twinges occurred that night while the patient was in bed. After a free interval of two weeks, discomfort returned, and for three months she had been completely incapacitated. The pain occurred particularly on exertion, but also at rest. Nitrites afforded some relief. There was no dyspnea or edema.

Examination.—The patient was slightly cyanotic. The heart was moderately enlarged. The sounds were clear and of fair quality. There was a musical systolic blow at the apex. The second aortic sound was hollow and slightly accentuated. The blood pressure was 160 systolic and 100 diastolic. The electrocardiogram showed inversion of T_1. The Wassermann test of the blood gave a negative result. The diagnosis was coronary sclerosis, hypertension and fibrosis of the myocardium. There was slight hyperesthesia to pressure in a localized area around the left nipple and over the left scapula. While she was in the ward, the patient continued to experience pain, even at rest.

A paravertebral injection of alcohol was given on March 27. The patient cooperated well, and there was no pain during the injection. On the following day, a definite Horner's syndrome was present. There was no cardiac pain and only slight tenderness over the site of injection. The temperature on the second day rose to 101.6 F. and the heart rate to 96. On the following day, the temperature was 102 F. and on the fourth day 101.6 F. At the end of a week the temperature was still 100 F. in the evening. Hypesthesia developed over the left side of the chest, in front, in back and in the axilla. As a result of the injection of alcohol, the pains in the precordia, the left arm and the left side of the neck disappeared. However, the patient continued to have attacks of moderate pain under the sternum, on the inner side of the right arm and in the right side of the neck. These attacks were accompanied by a definite rise in blood pressure, frequently to 200 mm. of mercury.

On the tenth day after injection, the patient complained of more intense pain, and the temperature rose to 102 F. The leukocyte count was 16,300, with 86 per cent polymorphonuclears. An electrocardiogram taken two days later showed that right bundle branch block had developed, with the usual marked inversion of T_1. This combination of observations afforded good evidence that coronary thrombosis had occurred, with infarction of the left ventricle. The patient complained of severe pain in the region of the apex, in the midsternal region and in

the right arm. She continued to have attacks and became unstable emotionally. Gradually, she began to improve and was finally able to sit in a chair.

It was difficult to estimate the severity of the pains, as she described them poorly and was always anxious and complaining. She was discharged on June 6, two and one-half months after injection. In this case, the picture was complicated by acute coronary obstruction. In spite of this occurrence, it should be noted that she was free from pain on the left side, but complained of substernal discomfort, radiating to the right arm.

On July 30 (four months after injection), she reported that there had been no recurrence of cardiac pain, though at times palpitation was annoying. She complained of a constant dull ache in the shoulders and arms, which was probably of neuritic origin, since roentgen examination revealed well marked arthritis of the cervical vertebrae. She was able to walk about the house and to go motoring. At this time the blood pressure was 210 systolic and 135 diastolic.

CASE 8.—*History.*—Arthur S., aged 58, an architect, was admitted to the hospital on March 7, 1930, complaining of paroxysmal pain in the sternal region and the epigastrium of five years' duration. The present illness began suddenly, when he was awakened early one morning by a terrific epigastric pain, sharply localized. This pain was relieved by a hypodermic injection. Shortly thereafter, while hurrying for a train, the pain recurred and made him stop. The attacks then increased as time went on and the amount of effort which he could make was greatly limited. During the early attacks, the pain had radiated to both arms, and it had been worse on the right side, but lately radiation was less marked. There had been no dyspnea or edema. For eight months, he had spent almost all of his time in bed. The slightest effort induced discomfort and he had a number of attacks while bedridden. He also suffered considerably from gaseous distention and constipation. The pain was always relieved by glyceryl trinitrate.

Examination.—Examination showed several badly infected teeth, which were promptly removed. The heart was not enlarged. The aorta was tortuous but not dilated, as shown in the roentgenogram. The blood pressure was 145 systolic and 80 diastolic. The electrocardiogram showed diphasic T_2 and sharply inverted T_3, together with notching of R_3. Roentgenograms of the gastro-intestinal tract showed a small duodenal ulcer, but there was no blood in the stools. The diagnosis was coronary sclerosis and duodenal ulcer. The ulcer appeared to be playing a minor rôle in the production of symptoms. However, the patient was put on a Sippy regimen for ulcer.

Operation.—On March 19, a paravertebral injection of alcohol was given. When the injection was made into the fourth thoracic root, the patient exclaimed, "That caused a pain in my heart just like the ones I always have." Injection into the other roots was unattended by discomfort. The procedure was well borne. Horner's syndrome did not appear. The patient had two attacks on the night following injection, but there was no fever or tachycardia at any time. On the third day, an area of anesthesia was noted in the back, over the lower portion of the scapula and in the left part of the midscapular and subscapular regions. There was no anesthesia in the axilla or in front. He continued to have mild attacks of pain, which were always relieved by the taking of glyceryl trinitrate, but these attacks occurred less frequently, and they were less disturbing. He left the hospital on April 19, as an ambulatory patient, one month after injection.

Course.—He was again seen on July 1 (three and one-half months after injection). He continued to have a sense of soreness in the upper part of the left

TABLE 1.—*Paravertebral Injections of Alcohol: Detailed Analysis of Thirty-Seven Cases*

Author	Case	Location of Pain	Duration of Attacks	Age and Sex	Diagnosis	Site of Injection*	Result	Degree of Relief†	Length of Follow-Up	Horner's Syndrome	Comments and Last Known Status
Swetlow (Am. Heart J. **1**: 393, 1926)	1	Precordial, radiating to left shoulder blade and down left arm; induced by slight effort	2 yr.	20 F	Syphilitic aortitis; aortic insufficiency	4-7 DL	Marked, though incomplete relief	P	6 mo.	?	Pain had persisted in spite of specific therapy; able to carry on increased activity; still having short fleeting pains
Swetlow and Schwartz (J. A. M. A. **86**: 1679 1926)	2	Retrosternal, penetrating anteroposteriorly; induced by effort	15 mo.	63 M	Coronary sclerosis	3-7 DL	Complete relief within 24 hours	C	4 mo.	?	Relief lasted 4 months, then pain recurred; given second injection
						1-4 DL	.....	C	6 mo.	?	Again completely relieved; had slight retrosternal pressure at times
Swetlow (Am. J. Surg. **9**: 88, 1930)	3	Precordial and in left interscapular region, both after effort and at rest	15 mo.	18 F	Mitral stenosis and insufficiency; cardiac insufficiency	3-9 DL	Moderate relief while in bed	P	3 mo.	?	Some pain persisted; died 3 months later of cardiac insufficiency; 60 per cent solution of alcohol used for injection
	4	Precordial, on effort and in bed, at night	4 yr.	61 M	Coronary sclerosis; coronary and cerebral thrombosis	1-2 DL	Marked relief	P	8 mo.	?	Almost complete relief; dyspnea and cyanosis on effort
	5	Precordial, radiating to left arm, on effort and in bed	2 yr.	65 M	Coronary sclerosis	2-5 DL	Pain disappeared	C	2 mo.	?	Pulmonary edema and dyspnea recurred; died suddenly after 2 months
	6	Precordial, radiating down left arm, on effort or at rest	6 yr.	72 M	Coronary sclerosis	1-5 DL	Great relief	P	3 mo.	?	Occasional fleeting precordial pain; able to increase activity; general condition improved because of relief from suffering
	7	Paroxysmal; on effort or at rest	3 yr.	60 F	Coronary sclerosis	1-9 DL	Complete relief of pain, though cardiac insufficiency persisted	C	6 days	?	Critically ill; died suddenly 6 days after injection
	8	Precordial, radiating to left arm, on effort and at night	5 yr.	62 M	Coronary sclerosis	1-9 DL	Great relief	P	4 mo.	?	Attacks of pain mild and fleeting; comfortable

White and White (J. A. M. A. **90**: 1999, 1928)	1	Left side of chest, radiating down left arm, on slight effort	3 yr.	54 M	Syphilitic aortitis; aortic insufficiency; hypertension; confined to bed	1-5 DL	Complete relief of left-sided attacks; recurrence of pain on right side	C (100%)	13 mo.	+	Attacks increased in frequency and severity in spite of specific therapy; second injection for attacks on right side
Richardson and White (Am. J. M. Sc. **177**: 161, 1929)		.....	.....	..	.....	1-5 DR	No relief of right-sided attacks	0	.....	..	Up and about; still having right-sided pain and occasionally substernal discomfort; unable to work
						1-5 DR	Improved	P (75%)	3 yr.	..	After third injection, rare right-sided pain; no left-sided pain
White (Arch. Neurol. & Psychiat. **22**: 304, 1929; Am. J. Surg. **9**: 98, 1930)	2	Substernal, radiating to interscapular region	4 yr.	60 M	Coronary sclerosis; aortic insufficiency; hypertension	2-6 DL	Relief for 2 weeks	P (slight)	5 wk.	+ (slight)	Pain recurred; had second injection after 5 weeks; attacks became milder
						1-3 DL	Pain milder	P (60%)	9 mo.	..	Resumed light work; died of coronary thrombosis 9 months later
	3	Precordial, radiating down left arm	3 yr.	53 F	Arteriosclerotic heart disease; hypertension	1-5 DL	Recurrence of pain after 6 days; little improvement	P (25%)	2¼ yr.	0	Still having several attacks each day; a nervous woman; up and about; painful hyperesthesia and paresthesia after injection; died of empyema after 2¼ years
	4	Substernal, radiating to both arms, worse on left	4 yr.	54 M	Arteriosclerotic heart disease	1-5 DL	Left-sided relief; still had right-sided attacks	P (100% on left)	2½ yr.	+	Pleural pain after injection; left-sided pain relieved; still had pain on right and dyspnea; resumed work as truck driver for 3 months; now having dyspnea
	5	"Typical angina"	1 yr.	59 M	Arteriosclerotic heart disease; hypertension; coronary thrombosis	1-5 DL	Partial relief	P (40%)	4 mo.	0	Pain less severe, but dyspnea increased; died suddenly 4 months later
	6	.....	.....	52 M	Hypertension; aortic insufficiency	1-5 DL	Complete relief at first; later slight recurrence	P (90%)	2¼ yr.	?	Comfortable and able to do light work
	7	.....	.....	58 M	Arteriosclerotic heart disease; hypertension	1-5 DL	Considerable relief	P (65%)	5 mo.	?	Resumed light work; comfortable
	8	.....	.....	68 M	Arteriosclerotic heart disease; hypertension; previous coronary thrombosis	1-5 DL	Partial relief at first, then recurrence	P (50%)	10 mo.	?	Up and about, but having daily attacks; died after 10 months of coronary thrombosis

* D indicates dorsal; C, cervical; R, right; L, left.
† C indicates complete; P, partial.

TABLE 1.—*Paravertebral Injections of Alcohol: Detailed Analysis of Thirty-Seven Cases—Continued*

Author	Case	Location of Pain	Duration of Attacks	Age and Sex	Diagnosis	Site of Injection*	Result	Degree of Relief†	Length of Follow-Up	Horner's Syndrome	Comments and Last Known Status
	9	.....	.....	47	Coronary sclerosis and thrombosis; hypertension	1-5 DL	Attacks relieved	C (100%)	2 mo.	?	Died of cardiac insufficiency 2 months later; had right-sided pain
	10	.....	....	51	Arteriosclerotic heart disease; coronary thrombosis; morphinism	1-5 DL	Relief until death	—	5 days	?	Another attack of coronary thrombosis 5 days later; died 3 weeks after injection
	11	.....	.....	56	Arteriosclerotic heart disease; hypertension	1-3 DL	Complete relief for 4 mo., then slight recurrence	C and P (100-90%)	7 mo.	?	Worked for 4 months; apoplexy; few mild attacks for 2 months
	12	.....	.....	57	Arteriosclerotic heart disease; hypertension; coronary thrombosis	1-4 DL	Much improved	P (90%)	4 mo.	?	Returned to light work; rare attacks
	13	.....	....	56	Arteriosclerotic heart disease; coronary thrombosis	1-3 DL	Complete relief	C (100%)	3 mo.	?	Continued to practice medicine
	14	.....	.....	54	Arteriosclerotic heart disease; coronary thrombosis	1-4 DL	Complete relief	C (100%)	2 wk.	?	Followed only 2 weeks
	15	.....	...	49	Arteriosclerotic heart disease	1-3 DL	Much improved	P (75%)	3 mo.	?	Planned to return to work
	16	.....	.....	57	Arteriosclerotic heart disease	1-4 DL	No relief	0	.....	?	Condition as before injection
	17	.....	.....	52	Syphilitic aortitis	1-4 DL	No relief	0	.....	?	Condition as before injection
	18	Upper right portion of chest and neck	.....	..	Syphilitic aortitis; aneurysm of ascending aorta	1-2 DR	Complete relief	C (100%)	3 mo.	?	Comfortable until death 3 months later, due to rupture of aneurysm
Sprague and Mixter (New England J. Med. **200**: 199, 1929)	1	Substernal	10 yr.	47 M	Coronary sclerosis and thrombosis; hypertension; obesity	1-5 DL	One mild attack 1 week after injection, then complete relief until death	C (100%)	2 mo.	?	Continued to have cardiac asthma; progressive cardiac failure terminated in death 2 months later; at operation, pain relieved after injection of third and fourth dorsal nerves

Cattell and Hurxthal (J. A. M. A. 92 : 1519, 1929)	1	Substernal and precordial, radiating down left arm	4 mo.	52 M	Coronary sclerosis	8 C/ 1-5 D\ L	Only occasional twinges for 5 mo., then coronary thrombosis and recurrence	P (10% for 5 mo.)	8 mo.	+	Worked for 5 months, then again incapacitated after attack of thrombosis
Levy and Moore	1	Left arm, traveling to precordium	8 mo.	36 M	Coronary sclerosis	1-5 DL	Pain entirely relieved	C	16 mo.	0	Radicular pain for 6 weeks; some dyspnea on effort; at work at his trade of baker; no cardiac pain
	2	Precordial, radiating to left shoulder and arm	4 yr.	54 M	Coronary sclerosis; diabetes mellitus	1-5 DL	Complete relief for 2 weeks, then recurrence	P	4 mo.	0	Great deal of radicular pain after operation; final result obscured by symptoms of cerebral arteriosclerosis; partial relief for 3 months
	3	Precordial	25 yr.	68 F	Coronary sclerosis; diabetes mellitus	1-5 DL	Relieved for 7 weeks	C (7 wk. then 0)	7 mo.	0	Relief at first, then recurrence; psychosis developed so that final result is in doubt
	4	Substernal, radiating to both sides of chest, to neck and jaws	8 wk.	35 M	Coronary sclerosis	1-5 DL	Complete relief	C	2 mo.	0	Pleural effusion developed after injection; disappeared from observation
	5	Precordial, radiating to left arm; in left eye	9 yr.	48 M	Coronary sclerosis	1-5 DL	No pain for 3 mo.; substernal oppression persisted	C (temporary)	6 mo.	+	Pleural effusion developed after injection; free from pain for 3 months; recurrence; never relieved from substernal oppression
	6	Precordial	8 yr.	68 M	Coronary sclerosis; diabetes mellitus; syphilis	1-5 DL	Slight relief for 1 month, then recurrence	0	7½ mo.	0	Severe radicular pain; slight temporary relief, a therapeutic failure
	7	Precordial, radiating to arms, ears and gums	3½ mo.	50 F	Coronary sclerosis; coronary thrombosis	1-5 DL	Left-sided relief for 10 days; pains under sternum and on right for a time, then complete relief	C	4 mo.	+	Coronary thrombosis 10 days after injection; recovery, followed by persistent, though less severe pain for a time, then complete relief
	8	Substernal and epigastric	5 yr.	58 M	Coronary sclerosis; duodenal ulcer	1-5 DL	Attacks much milder and less frequent	P	8 mo.	0	Greatly improved; after having spent 8 months in bed, he is now up and about, carrying on a fair amount of activity
	9	Substernal and precordial	2 yr.	47 M	Coronary sclerosis	1-5 DL	Attacks milder but still present	P	6 mo.	+	Partial relief; a person of meager intelligence

axilla, although it was not tender on deep pressure. The cardiac pain was much less frequent and less severe. The worst attack usually occurred in the morning, after breakfast, while shaving or bathing. The pain was invariably relieved by glyceryl trinitrate. He was much stronger and more active, and he was able to take motor rides and to resume a certain amount of social activity. He felt that he had been tremendously benefited by the injection and that he was continuing to improve. Roentgenograms of the gastro-intestinal tract showed that the duodenal ulcer had healed. On November 3 (eight months after injection), he reported continued progress and planned to resume part-time work.

CASE 9.—*History.*—Max S., aged 47, a Russian machinist, was first seen in the Cardiac Clinic on March 26, 1930. He complained of attacks of substernal pain and dyspnea on exertion for the past two years. The pain was of a twisting and squeezing nature, and it radiated toward the left arm and hand. The attacks gradually became more and more easily provoked, and lately they had been localized to the upper part of the sternum and the precordia. Nitrites afforded relief. Two years before, he had been at another hospital, where a diagnosis was made of manic-depressive psychosis in the depressive stage. He had not worked for two years.

Examination.—Roentgen examination showed moderate cardiac enlargement and moderate but diffuse dilatation of the aorta. The electrocardiogram showed inversion of T_1 and T_2. The blood pressure was 230 systolic and 120 diastolic. There were no signs of cardiac insufficiency. The diagnosis was hypertension, coronary sclerosis and fibrosis of the myocardium.

Operation.—On April 10, a paravertebral injection of alcohol was given. The patient was apprehensive and complained of some discomfort, but this was not severe and there were no manifestations of pleural irritation. On the evening of operation, the temperature rose to 101.2 F., and for a week thereafter it reached 100 or 100.2 F. in the afternoon. A definite Horner's syndrome developed. The patient had slight soreness at the site of the injections. He stated that the anterior part of the chest felt dead. Objectively, there was hypesthesia over the distribution of the first five thoracic nerves on the left side, in front and in back. There was no recurrence of the paroxysms of pain during his stay in the hospital, from which he was discharged on April 22, twelve days after injection.

Course.—The patient was seen on April 28 (eighteen days after injection). He had not used glyceryl trinitrate, but he stated that he had had pain in the back and over the precordial area. Horner's syndrome was still present, though less marked. The blood pressure was 224 systolic and 116 diastolic. There were occasional premature beats. Because of his depressed state, he was examined by a psychiatrist, who classified him as a constitutionally inferior individual. He was last seen on October 20 (six months after injection), and he still complained of pain all over the left side of the chest, together with a variety of other discomforts. It was almost impossible to appraise the degree of relief, because of the psychoneurotic state. The fact that he did not take glyceryl trinitrate indicated that he had been relieved at least in part. He did not seek work.

CLINICAL FEATURES

For detailed analysis, thirty-seven cases, including our own, are available, since Pletnew and Hesine [13] reported only collective results and Swetlow [23] noted full data in but eight cases. In table 1 are given

the essential facts, and in table 2 these facts have been summarized. The series is small and the periods of observation are relatively short, so that an attempt to draw general conclusions at this time would be premature. Certain features are worthy of brief comment.

It appears that age, sex, the character of the pain and the duration of symptoms prior to injection did not materially influence the outcome. A preponderant majority of the patients were elderly persons, with arteriosclerosis and coronary involvement. A fair estimate of the degree

TABLE 2.—*Paravertebral Injections of Alcohol: Summary of Thirty-Seven Cases in Which Data Were Available*

Number of injections		41
Left side	38	
Right side	3	
Etiologic type of cardiac disease:		
Arteriosclerotic		32
Syphilitic		4
Rheumatic		1
Relief obtained:		
Complete (49%)		18
Permanent	15	
(to death or last known status; 6 died within 6 months)		
Temporary	3	
Partial (43%)		16
Permanent	15	
Temporary	1	
None (8%)		3
Horner's syndrome:		
Present		7
Absent		8
Not noted		22
Untoward effects:		
Pleural effusion		2
Postoperative collapse		2
Bloody expectoration		1
Operative deaths		0
Duration of follow-up:		
Less than 6 months		20
6 to 12 months		12
1 to 2 years		1
2 to 3 years		4

of relief obtained may be made by averaging the results as recorded in forty-nine cases—twenty-two reported by Swetlow, eighteen from the Massachusetts General Hospital and nine of our own. Complete or almost complete relief was obtained in 51 per cent of the cases; improvement was noted in 34 per cent, and in 15 per cent the operation was a failure. According to these figures, then, 85 per cent of the patients have been benefited to some extent following injection. Even though the pain was not completely relieved, the attacks were described as milder and of less frequent occurrence. In some cases, relief was only slight and temporary; in others, the result was strikingly successful, as in case 1. Even partial relief, as in case 8, enabled the patient to return to limited activity, after having been confined to bed for most of the time during the eight months preceding operation. In one patient [8]

who received injections on the right side for dextral pain occurring after left-sided relief, a 75 per cent favorable effect was noted at the end of three years.

Unsuccessful results are probably due in part to faulty technic, with failure to infiltrate the proper area; in part, perhaps, to conduction of pain impulses by other unknown pathways, and possibly, also, in a smaller measure, to individual anatomic variations in the nerves concerned. No patient has died as the result of the operation, although several were critically ill. Two died of coronary thrombosis within three weeks.

In fifteen cases, specific mention is made concerning the occurrence of Horner's syndrome; it followed injection in seven instances. In our series of nine cases, it was observed three times. White and White [8] regarded this evidence of block of the upper dorsal sympathetic rami as a favorable sign, but our experience has not led us to stress its importance.

Frequent electrocardiograms were made in our cases, but no changes were observed that could be directly ascribed to the effects of the injections of alcohol. Swetlow [24] reached a similar conclusion.

Any evidence throwing light on the particular nerve roots concerned in the mechanism of cardiac pain has been eagerly sought. It was with particular interest that we noted the remarks of two patients at the time of injection. As an injection was made into the second dorsal root in one instance, and into the fourth dorsal in another, each patient exclaimed: "That gave me a pain in my heart just like the ones I have always had." In three of the cases at the Massachusetts General Hospital, attacks of pain occurred while the patients were on the table. In one case, Sprague and Mixter [9] observed that relief was promptly obtained after injections of alcohol were made into the third and fourth dorsal roots, whereas in the other two cases J. C. White [25] noted cessation of discomfort after injection was made into the third dorsal root. Pletnew,[13] who gave injections into the first three or four dorsal ganglions and sometimes also into the seventh and eighth cervical ganglions, recorded two similar experiences. Several of his patients stated that "the needle felt as though it had pierced the heart"; and from three to five minutes after injection, that "the heart disappeared." In one of his cases, while an injection was being made into the sixth and seventh cervical and the first three dorsal roots, signs of sudden cardiac weakness developed, for which strophanthin, camphor and caffeine were given. Multiplication of such observations should result in the accumulation of important data.

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24. Swetlow (footnote 7, third reference).
25. White (footnote 12, first reference).

The discomforts and untoward effects of paravertebral injections of alcohol have not been sufficiently emphasized. A majority of the patients (eight of nine in our series) complained bitterly of pain and hyperesthesia in the cutaneous distribution of the nerve roots into which injections were made. Pain began within twenty-four hours, after the effects of the procaine hydrochloride had worn off, and it lasted, in some of our patients, for as long as six weeks. This form of discomfort, which was probably due to intercostal neuritis induced by the alcohol, is apparently unavoidable, though distressing and discouraging. In a few cases, diathermy seemed to afford some relief. A moderate degree of fever (from 100 to 102 F.) was also observed in eight of our nine patients, and this symptom has been noted by others. This may persist for a week or ten days, and may well be due to necrosis of the tissues induced by the alcohol. Immediately following injection, and while still on the table, a patient of White and White [8] complained of severe pain on the left side and of faintness. He became pulseless for thirty minutes, but recovered within an hour. It was thought that the reaction might be due in part to the intravenous injection of procaine hydrochloride, though the pain was believed to be of cardiac origin. Mixter [11] stated that he thought that in one case he put a little alcohol into the pleura. The pain was severe and markedly increased by respiration, and for a short time he "was much disturbed as to the outcome."

In two of our cases, pleural effusion developed on the left side, which was confirmed by roentgen examination. The effusions were small, and they were accompanied by considerable pain and fever; slight leukocytosis was found in one instance. It is curious that the patient did not complain of pain or respiratory distress at the time the injections were made. Therapeutic aspiration was not necessary, and the fluid was absorbed within a week. During the night following operation, one patient [8] complained of mild pleural pain and raised some faintly blood-tinged sputum. In one of Swetlow's cases,[26] pneumothorax without effusion followed injection. Even with the utmost care in technic, the point of the needle rests very close to the pleural cavity. In the patients with effusion, it seems likely that the needle did not penetrate the pleura but caused irritation by contact of the alcohol with the outer surface.

In spite of these unpleasant and, at times, disturbing complications, the procedure of injection is comparatively safe in skilled hands. It has the disadvantage of being unaided by direct visual guidance. It is a measure designed to relieve pain, and it must clearly be understood that it does not, to the best of our knowledge, affect the basic pathologic state. Final judgment as to its value and limitations must be reserved until more cases have been observed over longer periods of time. For the present it should be employed only after carefully planned medical

26. Swetlow (footnote 7, fourth reference).

treatment has failed to alleviate intense suffering. Patients in whom pain has served as a danger signal of overexertion should be warned, if relieved of attacks, against exceeding the functional capacity of the heart.

SUMMARY

1. Based on fifty-seven cases reported in the literature and on nine that were personally observed, a critical analysis has been made of the results of paravertebral injections of alcohol for relief of cardiac pain.

2. The known facts concerning the nervous mechanisms involved in the transmission of cardiac pain have been briefly stated.

3. The technic of injection, as described, is relatively simple, but it requires skill acquired by experience on the cadaver. The procedure has the disadvantage of being unaided by visual guidance.

4. Of forty-nine cases in which adequate data are available, 51 per cent of the patients obtained complete or almost complete relief; improvement was noted in 34 per cent, and in 15 per cent the operation was a failure. Some patients were benefited only slightly and temporarily; in others, the result was strikingly successful. In one personal case, there has been complete relief from pain for sixteen months.

5. After injection, a majority of the patients suffered from hyperesthesia of the wall of the chest and painful intercostal neuritis in the segmental distribution of the nerves into which injections were made. At times the discomfort lasted for as long as six weeks, and it was apparently unavoidable. Many patients had fever following injection, which lasted from a few days to a week. In two of our cases, effusion into the left pleural cavity followed the injections.

6. Horner's syndrome was observed in seven of fifteen cases in which notes on this point are available. Its occurrence did not afford an index of the ultimate therapeutic result. It tended to disappear in the course of several weeks or months.

7. Paravertebral injection of alcohol offers a reasonably good hope of some relief to patients with paroxysmal cardiac pain. Final judgment as to its value and limitations must be reserved until more cases have been observed over longer periods. For the present, it should be tried only after carefully planned medical treatment has failed to alleviate intense suffering. The procedure is relatively safe, and so far it has been unattended by operative fatality. The method has a sounder physiologic basis and is less dangerous than cervical sympathectomy.

8. This form of therapy is entirely symptomatic. So far as we know, the basic pathologic condition is unaltered. Patients in whom pain has served as a danger signal of overexertion should be warned, if relieved from attacks, against exceeding the functional capacity of the heart.

Book Reviews

DAS ULCUS PROBLEM IM LICHTE MODERNER RÖNTGENFORSCHUNG. By PRIV. DOZ. DR. H. U. ALBRECHT, Oberarzt der medizinischen Universitäts-Klinik, Frankfurt, a-M. Paper. Price, 10 marks. Pp. 79, with 110 illustrations. Leipzig: Georg Thieme, 1930.

This monograph represents the result of combined clinical and roentgenologic study made on the subject of ulcer during a two year period. The author shows himself to be thoroughly familiar with the present-day trends in Germany, particularly with the anatomic studies of Konjetzny and the radiologic work of Berg. The study is divided into two phases, clinical and x-ray. The latter portion is excellently and adequately illustrated by means of films which emphasize the importance of the direct signs of ulcer.

During the period described, the author performed fluoroscopy on 1,527 patients with abdominal complaints of various kinds and found roentgen evidence of gastric or duodenal ulcer in 23.7 per cent. Ulcer was the most common abdominal lesion found. The ratio of duodenal to gastric ulcer was 5.8:1; the ratio of men and women afflicted was 80.99:19.01. Duodenal ulcer was found to be more frequent between the ages of 15 and 40, whereas gastric ulcer was somewhat more common after 40. The lesion apparently runs a more benign course in women than in men; hour-glass contracture of the stomach is more common in women. A familial story of ulcer was present in 21.2 per cent of the gastric lesions and in 30.5 per cent of the duodenal lesions. The author was unable to establish clear spring and autumn seasonal recurrences. He concludes by revising Moynihan's well known expression "In duodenal ulcer, the history is everything, the physical examination almost nothing" to read "In the diagnosis of peptic ulcer, the history and the x-ray are everything, the remaining clinical examinations almost nothing." The work is good, and it is carefully done. All students of gastro-enterology will find the book of interest.

A SYNOPSIS OF MEDICINE. By HENRY LETHEBY TIDY, M.A., M.D., B.CH. (OXON.), F.R.C.P. (LONDON), Physician to St. Thomas's Hospital. Fifth edition, revised. Cloth. Price, $6. Pp. 1,032, with index. New York: William Wood & Company, 1930.

The general arrangement of the book follows that of Osler and McCrae's "Principles and Practice of Medicine," and the special arrangement of headings and types is on the same system as in Groves' "Synopsis of Surgery"; the book was planned as a companion volume to the latter reference.

To quote from the preface, "It is hoped that the book may be of assistance to those who have to revise rapidly their knowledge of medicine in general or of some disease in particular; to the worried student whose final examinations are within sight and to the hurried practitioner from whose ken they have long passed, possibly even to the teacher with a lecture to prepare and to the examiner who, for the purposes of a vive voce, desires to renew for a brief period his knowledge of any of the essential details of medicine. The 'Synopsis' cannot replace a text-book to the student, and any attempt to make it do so will inevitably lead to failure."

Each disease is defined, and a short historical sketch is given; a general consideration as to etiology, mode of infection, bacteriology, morbid anatomy, symptomatology, types, complications and sequelae, diagnosis, prognosis and treatment, including prophylaxis, follows. By means of short summaries and special headings, the data that are of greatest importance have been clearly indicated. A full index has been provided.

THE PRINCIPLES AND PRACTICE OF MEDICINE: DESIGNED FOR THE USE OF PRACTITIONERS AND STUDENTS OF MEDICINE. Originally written by the late SIR WILLIAM OSLER, BT., M.D., F.R.S. Eleventh edition. Revised by THOMAS MCCRAE, M.D., Professor of Medicine, Jefferson Medical College, Philadelphia. Price, $7.50. Pp. 1,237. New York: D. Appleton & Company, 1930.

The eleventh edition of Osler's "Principles and Practice of Medicine," as revised by Dr. Thomas McCrae, hews to the line in presenting the fundamentals of medicine, principally adapted for the use of the medical student, but equally valuable for the general practitioner as a work of ready reference.

In revising the work, Dr. McCrae has been confronted by the difficult task of deciding how much of the new has earned the right to displace the old in keeping the work up to date and yet compressed into the confines of a not too bulky single volume. Traditionally, a definite and apparently successful effort has been made to retain everything that was characteristic of Osler. This text was one of the great interests of Osler's life, and the present edition has lost none of the original Osler flavor; this is its chief charm. Although much new material has been added, a proper perspective in describing the average picture of disease processes is preserved. Uniformity of style and clarity of expression particularly recommend the book for the use of the student. In all particulars, Osler's book remains the standard text, a position it has occupied for the past thirty-eight years.

A SYSTEM OF CLINICAL MEDICINE DEALING WITH THE DIAGNOSIS, PROGNOSIS, AND TREATMENT OF DISEASES FOR STUDENTS AND PRACTITIONERS. By DR. THOMAS D. SAVILL. Eighth edition. Price, $10. Pp. 1,017, with 167 illustrations. New York: William Wood & Company, 1930.

This edition has been completely revised under the supervision of Agnes Savill. Many of the chapters have been rewritten by Dr. Savill or by other English physicians, and an effort has been made to bring the material up to date.

The book is fairly comprehensive and covers in a brief way most matters of medical interest. The diseases are discussed from the standpoint of symptoms; the etiology, course of the disease, prognosis and treatment are dealt with later. On the whole, the descriptions of diseases are accurate and clearly written. Treatment seems to be a weak point throughout the book. Many drugs that have long been known to be of doubtful, if any, value are mentioned as being efficacious. There seems to be no critical discussion of therapy, and the advice is often misleading.

DER OPERIERTE MAGEN. By PROF. DR. HERMANN MEYER-BURGDORFF and DR. WALTER SCHMIDT, Göttingen. Paper. Price, 9.60 marks. Pp. 114, with 146 illustrations. Leipzig: Georg Thieme, 1930.

The literature on gastro-enterology heretofore has lacked an adequate presentation of the subject of postoperative gastric disturbances. In this monograph the authors describe their investigation of these disturbances in about 400 patients whose stomachs had been subjected to various operative procedures. The methods employed were those of careful clinical study and detailed roentgenologic examinations, the technic now in vogue on the Continent being used. This phase of the work has been done exceedingly well, for the task of obtaining definite roentgenologic information in a case in which an operation has been performed on the stomach is admittedly difficult. The numerous illustrations are excellent.

The various procedures of gastric surgical intervention are described in turn, sufficient consideration being given to the technic, the disturbances that may result and their clinical manifestations and the changes in the form of the stomach, in its secretion and in its motor function. Recurrent gastric carcinoma and recurrent peptic ulceration are discussed in detail. The bibliography is splendid.

The work will interest all students of gastric disease, internists, surgeons and roentgenologists.

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CONTENTS

PAGES

EXPERIMENTAL NEPHROTIC EDEMA. LOUIS LEITER, M.D., WITH THE TECHNICAL ASSISTANCE OF M. ELEANOR BLISH, S.B., AND DOROTHY R. GASTON, PH.D., CHICAGO 1

IS POLIOMYELITIS A DISEASE OF THE LYMPHATIC SYSTEM? MONTROSE T. BURROWS, M.D., PASADENA, CALIF. 33

EXPERIMENTAL EDEMA. SAMUEL A. SHELBURNE, M.D., DALLAS, TEXAS, AND WILLIAM C. EGLOFF, M.D., BOSTON 51

INSULIN: SOME EFFECTS ON NORMAL RABBITS PROTECTED FROM HYPOGLYCEMIA BY INGESTION OF DEXTROSE. ROBERT F. LOEB, M.D.; EMILY GUILD NICHOLS, AND BERYL H. PAIGE, NEW YORK 70

ACUTE TOXIC HEPATITIS (ACUTE YELLOW ATROPHY) DUE TO CINCHOPHEN: REPORT OF A CASE. K. K. SHERWOOD, M.D., REDMOND, WASH., AND H. H. SHERWOOD, M.D., KIRKLAND, WASH. 82

ESSENTIAL HYPERTENSION: THE DIASTOLIC BLOOD PRESSURE; ITS VARIABILITY. DAVID AYMAN, M.D., BOSTON 89

CINCHOPHEN OXIDATION TEST OF THE FUNCTION OF THE HEPATIC CELLS. S. S. LICHTMAN, M.D., NEW YORK 98

PHYSOSTIGMINE SALICYLATE IN THE TREATMENT OF EXOPHTHALMIC GOITER: OBSERVATIONS ON TWO HUNDRED CASES. ISRAEL BRAM, M.D., PHILADELPHIA 126

STAINING OF RETICULOCYTES BY BRILLIANT CRESYL BLUE: INFLUENCE OF SOLUTIONS OF SUBSTANCES. CLARK W. HEATH, M.D., AND GENEVA A. DALAND, S.B., BOSTON 133

PARAVERTEBRAL INJECTIONS OF ALCOHOL FOR THE RELIEF OF CARDIAC PAIN: A REVIEW OF EXPERIENCE TO DATE AND A REPORT OF NINE CASES. ROBERT L. LEVY, M.D., AND RICHMOND L. MOORE, M.D., NEW YORK 146

BOOK REVIEWS 169